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(54) Title: GALACTURONOSYL TRANSFERASES, NUCLEIC ACIDS ENCODING SAME AND USES THEREFOR

(57) Abstract: The invention provides an isolated nucleic acid molecule encoding the polypeptide having galacturonosyltra:

(GaIAT) activity. The GALA T 1 disclosed represents the first pectin biosynthetic glycosyltransferase gene isolated from the invention further provides 14 GALAT and 10 GALAT-like gene superfamily members. The identification of the GALA superfamily offers new opportunities to modulate pectin synthesis in vivo and in vitro by modulating the GALAT gene, for example, plants that produce modified pectins can be generated by altering the GALAT gene. Since modified pectins are presented to have improved agriculture. (57) Abstract: The invention provides an isolated nucleic acid molecule encoding the polypeptide having galacturonosyltransferase (GaIAT) activity. The GALA T 1 disclosed represents the first pectin biosynthetic glycosyltransferase gene isolated from plants. The invention further provides 14 GALAT and 10 GALAT-like gene superfamily members. The identification of the GALAT gene superfamily offers new opportunities to modulate pectin synthesisin vivo and in vitro by modulating the GALAT gene, for example, transgenic plants that produce modified pectins can be generated by altering the GALAT gene. Since modified pectins are predicted to affect plant growth, development, and plant defense responses, the transgenic plants are expected to have improved agricultural value. The modified pectins isolated from such transgenic plants are useful as gelling and stabilizing agents in the food, neutraceutical, and pharmaceutical industries. The expressed proteins, and variants thereof, of the GALAT superfamily are useful to produce in vitro modified pectins of commercial value.



# GALACTURONOSYLTRANSFERASES, NUCLEIC ACIDS ENCODING SAME AND USES THEREFOR

# CROSS REFERENCE TO RELATED APPLICATIONS

The present application claims benefit of United States Provisional Patent Application No. 60/445,539 filed February 6, 2003, which is incorporated in its entirety herein by reference to the extent not inconsistent herewith.

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#### **BACKGROUND**

This invention relates to plant physiology, growth, development, defense and, in particular, to plant genes, termed galacturonosyltransferases (GALATs), nucleic acids encoding same and the uses therefor.

Pectins are the most complex polysaccharides in the plant cell wall. They comprise 30-40% of the primary wall of dicots and non-graminaceous monocots, and  $\sim 10\%$  of the primary wall in the grass family. Pectins are a family of polysaccharides  $^{6,8,27}$  that include homogalacturonan (HGA) (Fig. 1), rhamnogalacturonan-I (RG-I) (Fig. 2) and rhamnogalacturonan II (RG-II) (Fig. 3) as well as xylogalacturonans (XGA) $^{32,34,38}$ and apiogalacturonans. While the specific structure of each of these polysaccharides differs as shown in Figs. 1-3, they are grouped into one family since they appear to be linked to each other in the wall and they each contain  $\alpha$ -D-galacturonic acid connected by a 1,4-linkage.

HGA is the most abundant pectic polysaccharide, accounting for ~55%-70% of pectin<sup>39</sup>. HGA is a linear homopolymer of  $\alpha$ 1,4-linked D-galactosyluronic acid that is partially methylesterified at the C6 carboxyl group and may be partially acetylated at O-2 and/or O-3<sup>8</sup> (Fig. 1). Some plants also contain HGA that is substituted at the 2 or 3 position by D-apiofuranose, the so-called apiogalacturonans (AGA)<sup>36,37</sup> and/or HGA that is substituted at the 3 position with D-xylose<sup>32-35</sup>, so-called xylogalacturonan (XGA). RG-II is a complex polysaccharide

that accounts for approximately 10-11% of pectin<sup>8,39</sup>. RG-II has an HGA backbone with four structurally complex side chains attached to C-2 and/or C-3 of the GalA<sup>8,27</sup> (Fig. 3). Rhamnogalacturonan I (RG-I) accounts for 20-35% of pectin<sup>39</sup> (Fig. 2). RG-I is a family of polysaccharides with an alternating [ $\rightarrow$ 4)- $\alpha$ -D-GalA-(1 $\rightarrow$ 2)-  $\alpha$ -L-Rha-(1 $\rightarrow$ ] backbone in which roughly 20-80% of the rhamnoses are substituted by arabinan, galactan, or arabinogalactan side branches<sup>6,8,30</sup>.

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Pectins are believed to have multiple roles during plant growth, development, and in plant defense responses. For example, pectic polysaccharides play essential roles in cell wall structure<sup>43</sup>, cell adhesion<sup>44</sup> and cell signaling<sup>45,46</sup>. Pectins also appear to mediate pollen tube growth<sup>47</sup> and to have roles during seed hydration<sup>48,49</sup>, leaf abscission<sup>50</sup>, water movement<sup>51</sup>, and fruit development<sup>47,8</sup>. Oligosaccharides cleaved from pectin also serve as signals to induce plant defense responses<sup>52,53</sup>. Studies of mutant plants with altered wall pectin reveal that modifications of pectin structure leads to dwarfed plants<sup>43</sup>, brittle leaves<sup>44</sup>, reduced numbers of side shoots and flowers<sup>54</sup>, malformed stomata<sup>44</sup> and reduced cell adhesion<sup>55</sup>.

Although pectins appear to have multiple roles in plants, in no case has their specific mechanism of action been determined. One way to directly test the biological roles of pectins, and to study their mechanisms of action, is to produce plants with specific alterations in pectin structure. This can be done by knocking out genes that encode the pectin biosynthetic enzymes. Such enzymes include the nucleotide-sugar biosynthetic enzymes and the glycosyltransferases that synthesize the pectic polysaccharides. Each glycosyltransferase is expected to transfer a unique glycosyl residue in a specific linkage onto a specific polymeric/oligomeric acceptor. To date, only five<sup>56-59,136</sup> of the more than 200 predicted wall biosynthetic glycosyltransferases have been funtionally identified at the gene level (i.e. enzyme activity of the gene product proven), and none of these have been shown to encode pectin biosynthetic enzymes.

Based on the known structure of pectin, at least 58 distinct glycosyl-, methyland acetyl-transferases are believed to be required to synthesize the family of

polymers known as pectin. As shown in the review by Mohnen, D. (2002) "Pectins and their Manipulation", G.B. Seymour et al., Blackwell Publishing and CRC Press, England, pp. 52-98, and Table I below, a minimum of 4-9 galacturonosyltranferases are predicted to be required for the synthesis of HGA, RG-I, RG-II and possibly for the synthesis of the modified forms of HGA known as XGA and AGA. The present invention relates to the identification of the first gene, GALAT1, encoding a galacturonosyltranferase and related genes thereto. The studies disclosed hereinbelow led the inventors to conclude that the gene GALAT1 UDP-GalA:Homogalacturonan  $\alpha$ -1.4known as encodes the enzyme Galacturonosyltransferase.

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**Table I.** List of galacturonosyltransferase activities predicted to be required for pectin biosynthesis<sup>9</sup>

Type of	Working <sup>1</sup>	Parent	Enzyme <sup>3</sup>	Ref for
GalAT	Number	polymer <sup>2</sup>	Acceptor substrate Enzyme activity	Structure
p-GalAT	1	HGA	*GalAα1→4GalA α1,4-GalAT	27
p-GalAT	2	RG-I	L-Rhaα1→4GalA α1,2-GalAT	27-29
p-GalAT	3	RG-II	L-Rhaβ1→3Api <i>f</i> α1,2-GalAT	30,31
D-GalAT	4	RG-II	L-Rhaβ1→3Api <i>f</i> β <i>1,3GalAT</i>	30,31
p-GalAT	5 ?⁴	RG-I/HGA	GalAα1→2LRha α1,4-GalAT	
p-GalAT	6?	RG-II/HGA	GalAα1→4GalA α1,4-GalAT	
p-GalAT	7?	XGA	GalAα1→4(Xyl β1→3)GalA <sup>5</sup> α1,4-GalAT	32-35
p-GalAT	8?	AGA	GalA $\alpha$ 1 $\rightarrow$ 4(Apif $\beta$ 1 $\rightarrow$ 2)GalA $\alpha$ 1,4-GalAT	36,37
p-GalAT	9?	AGA	GalA $\alpha$ 1 $\rightarrow$ 4(Apif $\beta$ 1 $\rightarrow$ 3)GalA $\alpha$ 1,4-GalAT	36,37

<sup>1</sup>Numbers for different members of the same groups are given based on pectin structure and on the assumption that HGA is synthesized first, followed by RG-I and RG-II. The numbers were given<sup>9</sup> to facilitate a comparison of the enzymes, but final numbering will likely correspond to the order in which the genes are identified.

<sup>2</sup>HGA: homogalacturonan; RG-I: Rhamnogalacturonan I; RG-II: Rhamnogalacturonan II; XGA: Xylogalacturonan; AGA; Apiogalacturonan.

<sup>3</sup>All sugars are D sugars and have pyranose rings unless otherwise indicated. Glycosyltranferases add to the glycosyl residue on the left\* of the indicated acceptor.

<sup>4</sup>The ? means the designated GalAT may be required if a different GalAT in the list does not perform the designated function.

25 <sup>5</sup>Glycosyl residue in the parenthesis is branched off the first GalA.

Over the years, membrane-bound  $\alpha$ 1-4galacturonosyltransferase (GalAT) activity has been identified and partially characterized in mung bean <sup>10,11</sup>, tomato <sup>12</sup>, turnip <sup>12</sup>, sycamore <sup>13</sup>, tobacco suspension <sup>2</sup>, radish roots <sup>5</sup>, enriched Golgi from pea <sup>7</sup>,

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Azuki bean<sup>14</sup>, Petunia<sup>15</sup>, and Arabidopsis (see Table II). The pea GalAT was found to be localized to the Golgi<sup>7</sup> with its catalytic site facing the lumenal side of the These results provided the first direct enzymatic evidence that the synthesis of HGA occurs in the Golgi. In in vitro reactions, GalAT adds [14C]GalA from UDP-[14C]GalA1,60 onto endogenous acceptors in microsomal membrane preparations to produce radiolabeled products of large molecular mass (i.e. ~105 kd in tobacco microsomal membranes² and ≥ 500 kd in pea Golgi<sup>7</sup>). The cleavage of up to 89% of the radiolabeled product into GalA, digalacturonic acid (diGalA) and trigalacturonic acid (triGalA) following exhaustive hydrolysis with a purified endopolygalacturonase confirmed that the product synthesized by tobacco GalAT was largely HGA. Thus, the crude enzyme catalyzes the reaction in vitro: UDP-GalAT + HGA(n)  $\rightarrow$  HGA(n+1) + UDP. The product produced in vitro in tobacco microsomes was ~ 50% esterified2 while the product produce in pea Golgi did not appear to be heavily esterified7. These results suggest that the degree of methyl esterification of newly synthesized HGA may be species specific and that methylesterification occurs after the synthesis of at least a short stretch of HGA. GalAT in detergent-permeabilized microsomes from azuki bean seedlings added [<sup>14</sup>C]GalA from UDP-[<sup>14</sup>C]GalA onto acid-soluble polygalacturonate (PGA) exogenous acceptors<sup>14</sup>. Treatment of the radiolabeled product with a purified fungal endopolygalacturonase yielded GalA and diGalA, confirming that the activity identified was a GalAT comparable to that studied in tobacco and pea. The azuki bean enzyme had a surprisingly high specific activity of 1300-2000 pmol mg<sup>-1</sup> min<sup>-1</sup>, especially considering the large amount (3.1-4.1 nmol mg<sup>-1</sup> min<sup>-1</sup>) of polygalacturonase activity that was also present in the microsomal preparations. As with the product made by tobacco, no evidence for the processive transfer of galactosyluronic acid residues onto the acceptor was obtained (see below).

**Table II.** Comparison of apparent catalytic constants and pH optimum of HGA- $\alpha$ 1,4-galacturonosyltransferases<sup>1,2</sup>

Enzyme <sup>2</sup>	Plant Source	Apparent K <sub>m</sub> for UDP-GalA (μM)	pH optimum	Vmax (pmol mg <sup>-</sup> <sup>1</sup> min <sup>-1</sup> )	Ref
GalAT <sup>1</sup>	mung bean	1.7	6.0	~4700	10
GalAT	mung bean	n.d.	n.d.	n.d.	61
GalAT	pea	n.d. <sup>5</sup>	6.0	n.d.	62
GalAT	pea	n.d.	n.d.	n.d.	7
GalAT	sycamore	770	n.d.	?	13
GalAT	tobacco	8.9	7.8	150	2
GalAT (sol) 3	tobacco	37	6.3-7.8	290	3
GalAT (sol) 3	Petunia	170	7.0	480	15
GalAT (per) <sup>4</sup>	Azuki bean	140	6.8-7.8	2700	14

<sup>1</sup>Adapted from ref 6.

<sup>5</sup> n.d.: not determined.

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GalAT can be solubilized from membranes with detergent<sup>3</sup>. Solubilized non-reducing end4 of exogenous HGA adds GalA onto the (oligogalacturonide; OGA) acceptors of a degree of polymerization of at least ten<sup>2</sup>. The bulk of the HGA elongated in vitro by solubilized GalAT from tobacco membranes<sup>3</sup>, or detergent-permeabilized Golgi from pea<sup>7</sup>, at roughly equimolar UDP-GalA:acceptor concentrations is elongated by a single GalA residue. These results suggest that solubilized GalAT in vitro acts nonprocessively, (i.e. distributively). The apparent lack of in vitro processivity of GalAT was recently confirmed by Akita et al. who, using pyridylaminated oligogalacturonates as substrates and high concentrations of UDP-GalA, showed that although OGAs can be elongated in a "successive" fashion with up to 10 GalA residues by solubilized enzyme from petunia pollen<sup>15</sup>, the kinetics of this response suggest a distributive mode of action. We have two working hypotheses as to why GalAT in vitro does not appear to act processively. One hypothesis is that the solubilized enzyme or the enzyme in particulate preparations does not have the required factors, or is not present in the required complex, to act processively. An alternative hypothesis is that for a Golgi-localized enzyme that synthesizes a complex polymer in a confined

<sup>&</sup>lt;sup>2</sup> Unless indicated, all enzymes are measured in particulate preparations.

<sup>&</sup>lt;sup>3</sup> (sol): detergent-solubilized enzyme.

<sup>&</sup>lt;sup>4</sup> (per): detergent-permeabilized enzyme.

internal cellular compartment, such as GalAT, with sufficiently high concentrations of substrate, it would not necessarily be advantageous for the enzyme to act processively. In fact, the reaction velocity could be hindered under such conditions if the enzyme were processive<sup>65</sup>.

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The apparent kinetic constants and pH optimum for the characterized GalATs are shown in Table II. We have performed additional kinetic studies in tobacco and radish that suggest that solubilized and membrane bound GalAT may have unusual apparent biphasic kinetics. We tested Vo for radish GalAT at 2 µM to 80 mM UDP-GalA and obtained a biphasic curve (Fig. 4), suggesting that the kinetics of GalAT, at least in the membrane and soluble fractions, are complex. Comparable results were also obtained for the solubilized radish and tobacco enzyme. The initial Vo vs [UDP-GalA] curve was hyperbolic and appeared to reach an initial maximum Vo of ~ 300 pmol mg<sup>-1</sup> min<sup>-1</sup> at ~1 mM UDP-GalA, confirming previous results reported for tobacco<sup>2,3</sup>. However, at ≥ 2 mM UDP-GalA there was a second hyperbolic increase in GalAT activity that reached a maximum of ~2-4 nmol min<sup>-1</sup> mg<sup>-1</sup> with ~20 mM UDP-GalA. In crude enzyme preparations it was not possible to determine the basis for the unusual kinetics. One possibility is that two GalATs were present, one with a low Km and one with a high Km. Another possibility is that UDP-GalA is both a substrate and an allosteric regulator of GalAT. Alternatively, a more "trivial" explanation is that at low substrate concentrations the kinetics of GalAT were effected by a catabolic enzyme (e.g. a phosphodiesterase) in the enzyme preparation.

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As a first step towards elucidating the role of galacturonosyltransferase (GALAT) in pectin synthesis, the inventors herein identified an *Arabidopsis* gene encoding alpha1,4- galacturonosyltransferase 1 (GALAT1). The database searches using the amino acid sequence of the GALAT1 identified fourteen additional *GALAT* family members and ten *GALAT*-like genes. The identification of these genes and the availability of the sequence information allow the characterization of the enzyme, the use of these genes to produce mutated enzymes *in vivo* and *in vitro*, and transgenic plants producing modified pectins, and

studies of the role of a specific GalAT in pectin synthesis. The advantages of the present invention will become apparent in the following description.

#### SUMMARY OF THE INVENTION

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The present invention provides an isolated nucleic acid molecule encoding the polypeptide having galacturonosyltransferase (GalAT) activity. The GALAT 1 disclosed herein represents the first functionally proven pectin biosynthetic glycosyltransferase gene isolated from plants. Also provided are additional 14 GALAT gene family members and 10 GALAT-like genes predicted to have galacturonosyltransferase activity. The identification and availability of the nucleic acid molecules as a member of the GALAT gene superfamily offer new opportunities to modulate pectin synthesis in vivo and in vitro by modulating the GALAT gene using various art-known recombinant DNA technology. For example, transgenic plants that produce modified pectins of desired properties can be generated by manipulating the gene encoding the GALAT protein i.e., mutating the gene including coding and non-coding sequences, silencing the gene by RNAi approach, or by administering a composition that would affect the GalAT activity in the plant. Since modified pectins are predicted to affect plant growth, development, and plant defense responses, the transgenic plants thus modified are expected to have improved agricultural value. The modified pectins can be isolated from such transgenic plants according to the art-known methods and serve as gelling and stabilizing agents of improved properties in the food, neutraceutical, and pharmaceutical industries.

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The inventors herein identified the first gene, *GALAT1*, which encodes a pectin biosynthetic enzyme by employing a partial purification-tandem mass spectrometry approach combined with a search of the *Arabidopsis* gene/protein database. Two genes, designated JS33 and JS36 herein, were identified as present only in the GalAT-containing fractions. As demonstrated hereinbelow, the expressed protein from the nucleic acid sequence of JS36 indeed exhibits the predicted GalAT enzymatic activity.

A standard protein blast and a PSI Blast of the NCBI protein database using the GALAT1 (JS36) amino acid sequence revealed that *GALAT*1 is a member of a 15 member *GALAT* gene family in *Arabidopsis*. The genes selected for this family have at least 30% amino acid identity and at least 50% amino acid similarity based on the PSI Blast. The database search using the GALAT1 sequence further identified 10 *GALAT*-like genes as shown in Table IV. The genes disclosed herein, fifteen *GALAT* genes and ten *GALAT-like* genes thus represent the *GALAT* gene superfamily members.

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The availability of the amino acid and nucleotide sequences of the *GALAT* gene superfamily members makes it possible to identify other *GALAT* homologs in other plants. The nucleotide and amino acid sequences of the *GALAT* genes can also be used to generate specific antibodies for the protein.

# BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 shows the trimeric region of homogalacturonan (HGA). HGA is a linear homopolymer of alpha-1,4-linked galacturonic acid that may be methylesterified at C6 and acetylated at O2 or O3. Substituted galacturonans, such as RG-II and apiogalacturonan, have an HGA backbone.

Fig. 2 shows the representative structure of rhamnogalacturonan I (RG-I). RG-I has an alternating [ $\rightarrow$ 4)-alpha-D-GalpA-(1 $\rightarrow$ 2)-alpha-L-Rhap-(1 $\rightarrow$ ) backbone in which roughly 20-80% of the rhamnoses are substituted by arabinans, galactans, or arabinogalactans.

Fig. 3 shows the representative structure for rhamnogalacturonan II (RG-II). RG-II has a backbone of 1,4-linked alpha-D-GalpA residues. GalA residues are also present in RG-II side chain A.

Fig. 4 illustrates the GalAT kinetics in radish microsomal membranes. Radish microsomal membranes (60-80  $\mu g$  protein) were incubated with 70  $\mu g$  of OGA (DP 7-23) and the indicated concentrations of UDP-GalA. Each reaction

contained a small concentration of UDP-[ $^{14}$ C]GalA (2-3.6  $\mu$ M) with larger amounts of nonradioactive UDP-GalA. The precipitated reaction products were measured by liquid scintillation counting. The data are the averages of duplicate samples from three separate experiments. The Y axis is specific activity (pmole min $^{-1}$ mg $^{-1}$ ).

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Fig. 5 shows the outline of the strategy to identify the gene for GalAT. The sequenced *Arabidopsis* genome allowed the use of a function-based partial purification-mass spectrometry approach to identify the putative galacturonosyltransferase genes. The sample analyzed in each lane is as follows: lane 1: homogenate, lane 2: total membranes, lane 3: solubilized proteins, lane 4: initial anion exchange purification step.

Figs. 6A and 6B show the results of RT-PCR experiments; 6A shows the results of JS33, JS36, and JS36L (a GalAT family gene with 63% identity to JS36) using *Arabidopsis* flower (F), root (R), stem (S), and leaf (L) RNA, and B shows the RT-PCR control using *Arabidopsis* actin gene in the same tissues.

Fig. 7 is a schematic representation of the transmembrane spanning region and the conserved amino acids in the *Arabidopsis thaliana GALAT* gene family. The relative position of the strictly conserved residues among the members of the proposed *GALAT* family is numbered as for JS36 (i.e., GALAT1). The striped region from residues 22-44 represents the predicted transmembrane region.

(At3g61130) **JS36** has demonstrates that recombinant Fig. 8 Human embryonic kidney cells galacturonosyltransferase (GalAT) activity. (HEK293) were transiently transfected with the pEAK vector alone, or with pEAK vector containing the truncated versions of JS33 or JS36. Total media (1); protein immunoabsorbed from the medium using anti-HA epitope: Protein A Sepharose (2); and protein immunoabsorbed from the medium using anti-HA epitope:Protein G Sepharose (3) were tested for GalAT activity. Data are the average [14C]GalA incorporated into product from duplicate reactions from three separate experiments.

Fig. 9 shows the relationship of the *Arabidopsis* GalAT superfamily including the GalAT family and the GalAT-like family. The Neighbor-Joining Tree is based on a sequence alignment generated by ClustalX.

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#### DETAILED DESCRIPTION OF THE INVENTION

In general the terms and phrases used herein have their art-recognized meaning, which can be found by reference to standard texts, journal references and contexts known to those skilled in the art. The following definitions are provided to clarify their specific use in the context of the invention.

In the present application, the designation, "GALAT", is used to denote the gene for galacturonosyltransferase, "GALAT" is used to denote the protein encoded by the gene, and "GalAT" is used to indicate galacturonosyltransferase enzyme activity.

The term, "polypeptide", is used herein interchangeably with "protein" to indicate a product encoded by a given nucleic acid.

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The terms, "identity" or "similarity" as used herein, are intended to indicate the degree of homology between the two or more nucleic acid or amino acid sequences. The degree of identity or similarity can be determined using any one of the computer programs that are well known in the art. The National Center for Biotechnology Information (NCBI) website on the internet provides detailed description and references necessary for this subject. Also see Karlin and Altschul (1993) *Proc. Natl. Acad. Sci. USA* **90**:5873-5877; Altschul *et al.* (1997) *Nucl. Acids. Res.* **25**:3389-3402. In the present application, the percent amino acid identity and similarity among the *GALAT* gene family and *GALAT*-like gene family members were carried out using the NCBI Pairwise Blast and Matrix Blosum62 using the GALAT1(JS 36) amino acid sequence.

A "corresponding" nucleic acid or amino acid or sequence of either, as used herein, is one present at a site in a GALAT molecule or fragment thereof that has the same structure and/or function at a site in another GALAT molecule, although the nucleic acid or amino acid position may not be identical.

The term "gene" is used herein in the broadest context and includes a classical genomic gene consisting of transcriptional and/or translational regulatory sequences and/or a coding region and/or nontranslated sequences (i.e., introns, 5'- and 3'-untranslated sequences), or mRNA or cDNA corresponding to the coding regions (i.e., exons) and 5'- and 3'-untranslated sequences.

The meaning of a "homolog" as used herein is intended to indicate any gene or gene product which has a structural or functional similarity to the gene or gene product in point. For example, a new homolog of a given *GALAT* gene can be identified either by a database search using the amino acid or nucleic acid sequences of a given *GALAT* gene or by screening appropriate cDNA or genomic libraries according to the art-known methods.

An "expression vector" as used herein, generally refers to a nucleic acid molecule which is capable of expressing a protein or a nucleic acid molecule of interest in a host cell. Typically, such vectors comprise a promoter sequence (e.g. TATA box, CATTbox, enhancer etc) fused to a heterologous sequence (i.e., a nucleic acid of interest), sense or antisense strand, followed by a transcriptional termination sequence, a selectable marker, and other regulatory sequences necessary for transcription and translation of the nucleic acid of interest. A plant expressible promoter is a promoter comprising all the necessary so called regulatory sequences for transcription and translation of a gene of interest in plants. The linkage between the heterologous sequence and the regulatory sequences (e.g., promoter) is "in operable linkage" when a desired product can be made from the heterologous sequence under the control of the given regulatory sequences. An "expression vector" is often used interchangeably with an "expression construct" in this sense.

The term "transgenic plant" as used herein refers to a plant that has been transformed to contain a heterologous nucleic acid, i.e., a plant expression vector or construct for a desired phenotype. The transgenic plant is intended to include whole plant, plants parts (stems, roots, leaves etc.) or organs, plant cells, seeds, and progeny of same. The transgenic plant having modified pectin of the present application is one that has been generated by manipulating the gene encoding the GALAT protein. This can be achieved, for example, by mutating the gene, silencing

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the gene by RNAi approach, or by knocking out the gene. The transgenic plants of the invention are predicted to have properties such as changes in organ and plant size, water transport properties, ease of removal of leaves and fruits via effects on abscision, pollen development and release, fruit ripening, root mucilage production, root growth, root cell cap production and separation, stem elongation, shoot growth, flower formation, tuber yield, defense responses against pathogens, and stomata opening<sup>8</sup>. Thus, the invention provides new means of improving plants of agricultural value. The "modified" pectins are those that exhibit structures and properties (e.g., gelling and stabilizing) different from those of the pectins naturally present in plants. Since galacturonic acid is a component of each of the pectic polysaccharides (i.e. HGA, RG-I, RG-II and XGA), a modification of the GalATs that add the specific GalAs into the specific polysaccharides is expected to modify the unique polymers. Such changes in pectin structure would affect multiple pectin properties including ionic interactions between HGA regions, gelation properties, dimer formation of RG-II molecules, length and degree of branching of RG-I, and side branch structure of RG-II. Such modifications are predicted to not only affect the biological function of pectin in plants, and the chemical and biological properties of pectin extracted and used by the food and cosmetic industries, but also properties that affect the use of pectin as a biopolymer for industrial processes, as a drug delivery polymer, and pectins of medicinal and neutraceutical properties in human and animal health.

The term "mutation" as used herein refers to a modification of the natural nucleotide sequence of a nucleic acid molecule made by deleting, substituting, or adding a nucleotide(s) in such a way that the protein encoded by the modified nucleic acid is altered structurally and functionally. The mutation in this sense includes those modifications of a given gene outside of the coding region.

The present invention provides polypeptides and nucleic acids encoding the polypeptides belonging to a family of the pectin biosynthetic enzyme, galacturonosyltransferase (GALAT). Pectins have been implicated in a broad range of plant growth phenomena including pollen tube growth<sup>47</sup>, seed hydration<sup>48-49</sup>, leaf abscission<sup>50</sup>, water movement<sup>128</sup>, and fruit development<sup>8</sup>. In addition, pectic oligosaccharides serve as signals<sup>45</sup> during plant development<sup>45</sup> and induce plant

defense responses<sup>52-53</sup>. Mutant studies have shown that altered pectin structure leads to dwarfed plants<sup>43</sup>, brittle leaves<sup>44</sup>, reduced numbers of side shoots and flowers<sup>129</sup>, and plants with reduced cell-cell adhesion<sup>130, 55</sup>. Therefore, the present invention provides the molecular and biochemical tools needed to identify additional glycosyltransferases involved in branching of the backbones, and would allow the generation of plants with altered pectin structure. While the 25 genes disclosed herein represent only ~0.1% of the ~28,000 genes in *Arabidopsis*, they are some of the most difficult genes to identify and characterize because of a lack of commercially available acceptor substrates and activated glycosyl donor substrates.

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The GALAT1 gene has high sequence similarity to proteins expressed in other plants, thus using the sequences disclosed herein, a person of ordinary skill in the art can identify other pectin biosynthetic genes (i.e. homologs) in other plant species, including agriculturally important plants. Since pectin of very similar structure is present in the walls of all flowering plants and gymnosperms, the identification of functional pectin biosynthetic genes will greatly facilitate the engineering of plants with modified pectin and with altered growth characteristics, some of which are expected to yield plants of increased agronomical value. In addition, mutant plants with defined changes in pectin synthesis can allow the dissection of the biological role of each pectic component in plants. The pectin biosynthetic genes provide valuable tools for understanding mechanistically how pectin is synthesized. The glycosyltransferase-specific antibodies that can be generated using the sequences disclosed herein are also within the scope of the invention and allow the process of pectin assembly in the Golgi to be elucidated. A complete understanding of such a polysaccharide cellular trafficking process is unknown in any biological system.

Pectin is found in fruits and vegetables and is used as a gelling and stabilizing agent in the food industry. Pectin has been shown to have multiple beneficial effects on mammalian systems and on human health including the inhibition of cancer growth and metastasis, inhibition of cancer metastasis by binding of pectic oligosaccharides to cell surface receptors of cancer cells (US5834442, US5895784), immunomodulatory effects and stimulation of tumor

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necrosis factor by macrophages (EP03983113), interaction with mucous cell lining of the duodenum and the prevention of ulcers (US4698229, US6024959); and anticomplementary activity 125. Many cancer cells have specific carbohydrate-binding protein molecules on their cell surfaces called galectins (galactoside-binding lectins). Galectins aid in cellular interactions by binding to beta-galactose linked molecules on neighboring cancer cells. Galectin-3 is a multifunctional lectin that is involved in tumor cell adhesion, metastasis and cancer progression. Blocking galectin-3 expression in malignant human breast, papillary and tongue carcinoma cells led to reversion of the transformed phenotype and suppression of tumor growth in nude mice 117-119. A pH-modified citrus pectin is suggested to block binding of galectins and inhibit tumor cells adhesion. Pienta et al. 127 showed that feeding of pH-modified pectin to rats caused a reduction in metastasis of prostate Similarly, oral administration of pectin to mice carrying colon tumors. reduced tumor size compared to control animals<sup>114</sup>, reduced metastatic colonization of B16-F1 melanoma in the lung 120-121 and reduced human breast and colon carcinoma growth, angiogenesis, and metastasis<sup>125</sup>. When prostate cancer patients were fed pH-modified citrus pectin, a 30% lengthening in prostate specific antigen (PSA) doubling time was observed in 57% of the patients 122. progression of prostate cancer is evaluated based on the time that it takes for the PSA to double, the above observations suggested that pectins may reduce tumor size. It has also been shown that fruit-derived pectins inhibit the interaction of fibroblast growth factor 1 (FGF1) to its receptor (FGFR1)<sup>123</sup>. Defects in the FGF signal transduction system are known to disturb cellular regulatory processes resulting in cancer, cardiovascular disease and diabetes mellitus. The availability of the gene(s) encoding galacturonosyltransferase allows the modification of neutraceutical or pharmaceutical pectins to provide pectins with novel cell and molecule binding activities and thus, with novel and specified anticancer and other physiological activities.

In order to identify a gene(s) involved in pectin biosynthesis, the inventors used a partial purification-tandem mass spectrometry approach to identify putative *GALAT* genes from *Arabidopsis* (see Fig. 5 for strategy). GalAT from *Arabidopsis* was partially purified from detergent-solubilized enzyme by sequential passage over two or more of the following resins: cation exchange resin SP-Sepharose,

reactive green 19 resin, reactive blue 72 resin, reactive yellow 3 resin, and UDP-agarose. Proteins obtained from selected fractions from these columns were treated with trypsin to generate peptides, and the amino acid sequence of the peptides identified by liquid chromatography-tandem mass spectrometry. The amino sequence thus generated was used to screen the *Arabidopsis* gene/protein database. Thirty unique proteins were solely identified in the GalAT-containing fractions (i.e. not present in fractions not containing GalAT activity). Among the 30 unique proteins that co-purified with GalAT activity, two proteins (designated JS33 and JS36) were initially identified as *Arabidopsis* putative GALAT proteins/genes based on their having at least one predicted transmembrane domain and since they contained a predicted glycosyltransferase domain (see CAZy database; <a href="http://afmb.cnrs-mrs.fr/CAZY/index.html">http://afmb.cnrs-mrs.fr/CAZY/index.html</a>).

These two genes, along with another *Arabidopsis* gene with high sequence similarity to JS36 (designated JS36L for JS36-like) (see below) were either cloned by RT-PCR (JS36) using mRNA from *Arabidopsis* flower and stem tissue, or a cDNA clone was obtained from the Arabidopsis Biological Resource Center (JS33 and JS36L). The proteins encoded by these genes each have a predicted single transmembrane domain (Table III). The genes were truncated to remove their N-terminal region including all or most of the predicted transmembrane domain (see Table III), and the truncated genes were inserted into a mammalian expression vector pEAK10 (Edge BioSystems as modified by Kelley Moremen Iab, CCRC) containing an N-terminal heterologous signal sequence (targeting the protein for secretion into the medium), a polyhistidine (HIS) tag, and two influenza hemagglutenin (HA) epitopes (useful for immunoabsorption).

**Table III.** Predicted characteristics of JS36, JS33 and JS36L proteins. Predictions were made using information from the NCBI database and the SOSUI (Classic & Membrane Prediction program) at BCM Search Launcher site (<a href="http://searchlauncher.bcm.tmc.edu/seq-search/struc-predict.html">http://searchlauncher.bcm.tmc.edu/seq-search/struc-predict.html</a>).

Gene	NCBI protein ID	# amino acids	MW (kd)	pl	Predicted transmembrane domain	Truncated protein
At3g61130 (JS36)	NP_191672	673	77.4	9.95	<sup>N</sup> 22-44 <sup>C</sup>	N42-673 <sup>C</sup>
At2g38650 (JS33)	NP_565893	619	69.7	8.63	<sup>N</sup> 23-45 <sup>C</sup>	N44-619 <sup>C</sup>
At5g47780 (JS36-like)	NP_568688	616	71.1	9.26	<sup>N</sup> 6-22 <sup>C</sup>	N26-616 C

The truncated forms of JS33, JS36 and JS36L, and the vector alone, were transiently expressed in human embryonic kidney cells (HEK293 cells) for 46 hours. Since the translational fusion proteins constructed contained two copies of the HA epitope, the culture medium was collected and a portion was treated with a mouse anti-HA IgG1 bound either to Protein A Sepharose or Protein G Sepharose. The immunoadsorbed protein was assayed for GalAT activity using UDP-[14C]GalA and a mixture of OGA acceptors. Figure 8 shows that the JS36 construct expressed a protein exhibiting GalAT activity. These studies establish that JS36 is a GalAT and thus we designated the gene *GALAT1*.

As mentioned above, analysis of the amino acid sequence of GALAT1 shows that the expressed protein contains one transmembrane domain. This is in agreement with the GalAT activity being membrane bound in all species tested (see Mohnen *et al.* (2002)<sup>9</sup>. Furthermore, the predicted topology of GALAT1 is that of a type-II membrane protein, in agreement with our previous determination that the catalytic site of pea GalAT lies in the lumen of the Golgi. Type-II membrane proteins have a short N-terminal cytosolic tail, a transmembrane region, a stem region, and a C-terminal catalytic domain<sup>16</sup>.

GALAT1 is a member of the Glycosyltransferase Family 8 in the CAZy database [database of putative and proven carbohydrate modifying enzymes that currently contains 61 different proposed glycosyltransferase families (http://afmb.cnrs-mrs.fr/CAZY/index.html) $^{66,67}$ ]. The presence of GALAT1 in Family 8 is in agreement with our demonstrated activity of GALAT1 as an  $\alpha$ 1,4-galacturonosyltransferase, since Family 8 is a family of proposed retaining glycosyltransferases and GALAT1 is a retaining enzyme, i.e., the  $\alpha$ -configuration in the substrate UDP- $\alpha$ -GalA is retained in the product  $\alpha$ 1,4-linked-galacturononan (HGA).

GALAT is expressed in multiple *Arabidopsis* tissues at multiple times during development. We base this on our RT-PCR analysis of RNA from *Arabidopsis* flower, root, stem and leaf tissue (Figs. 6A and 6B) showing that GALAT1 is expressed in all these tissues, and based on the 18 EST entries for this gene in the TAIR database (<a href="http://www.arabidopsis.org/">http://www.arabidopsis.org/</a>) indicating that GALAT1 is expressed in developing seed, green siliques, roots and above ground organs.

# Identification of the GALAT1 Gene Family

A standard protein blast and a PSI Blast of the NCBI protein database using the GALAT1 (JS36) amino acid sequence reveal that *GALAT1* is a member of a at least 15 member *GALAT* gene family in *Arabidopsis* (see Table IV). The genes selected for this family have at least 30% amino acid identity and at least 50% amino acid similarity based on the PSI Blast. We further compared these genes along their entire coding sequences with JS36 using a Pairwise BLAST (Table IV) and show that this family of genes has at least 34% identity and at least 52% similarity to JS36 in the portion of the genes C-terminal to the membrane spanning domain. This identity is comparable to the 37-54% identity shared among the proposed ten member *Arabidopsis* fucosyltransferase gene family (AtFU1-10)<sup>71</sup>.

Mutant studies provide further evidence that the GalAT family encodes GalATs involved in pectin synthesis. We recently used seed received from Arabidopsis T-DNA mutant collection (SIGnAL; http://signal.salk.edu/cgi-bin/tdnaexpress) to identify and generate six homozygous Arabidopsis GalAT family T-DNA insert mutant lines of several members of the GalAT family. We found that one GalAT family gene At1g06780, when mutated, produces leaves with cell walls that contain reduced amounts of galacturonic acid. Specifically, analysis of walls from homozygous mutant line 073484 revealed that the walls had an 18% reduction in GalA and a concomitant increase in glucose. None of the other sugars changed. Of the three available At1g06780 T-DNA insert lines, no homozygous seed was recovered from mutants where the T-DNA was inserted into an exon. Rather, seed recovered from such lines had a reduced germination rate. In line 073484. however, the T-DNA is inserted in the 5'-UTR, suggesting that it may have a leaky phenotype. The results are consistent with gene At1g06780 encoding a GalAT and with the identification of the gene family as a GalAT gene family. The GalA content of the walls of another Arabidopsis mutant (Quasimodo) is reduced by 25% and these plants exhibit decreased cell adhesion<sup>55</sup>, characteristics consistent with the Quasimodo gene encoding a GalAT. Quasimodo has 53% amino acid identity and 72% similarity to GALAT1 and the gene affected in Quasimodo (At3g25140) is a member of our proposed GalAT family. There is, however, at present no direct enzymatic evidence that the protein encoded by Quasimodo is a functional GalAT.

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The conserved amino acids in the GALAT gene family are shown in Fig. 7. Glycosyltransferases are expected to contain one or more carboxylates at the catalytic site. At least one of the carboxylates is expected to coordinate a divalent cation associated with the nucleotide-sugar. In many glycosyltransferases the metal coordination involves two carboxylates that are often present as DDx, xDD, or DDD (the so-called "D(x) D" motif)<sup>72</sup>.

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A PSI Blast against GALAT1 gene (JS36) further identified10 genes that have high sequence identity (23-29%) and similarity (41-51%) to GALAT1 and form a tight cluster of highly similar genes (55-66% identity/67-77% similarity). A Neighbor Joining Tree of our proposed Arabidopsis GalAT Superfamily (i.e. the proposed GALAT family and the GALAT-Like family), based on a sequence alignment generated by ClustalX<sup>128</sup>, is shown in Fig. 9. The 10 GALAT-like genes are all significantly smaller, lacking ~200 amino acids in comparison with the GALAT family. Nonetheless, they appear to be targeted to the secretory pathway based on annotation of the genes at the Arabidopsis Information Resources. All 10 genes appear to be expressed in Arabidopsis, since they are represented by one or more ESTs in the Arabidopsis EST collection. The GALAT-like genes also contain some of the same conserved residues as the GalAT family, namely D-D----D--L (the predicted "D(x) D" motif) and L------H--G--KPW. We group the 10 GALAT-like genes into a family that encode GalATs directly involved in pectin synthesis or GalATs with, as yet, unidentified glycosylating function.

Gene	NCBI protein	EMBL	% Identity	% Similar amino
	ID	protein #	(#aa identical/#aa)	acids (aa/aa)
GalAT-Family				
***At3g61130 (GALAT1;	NP_191672	Q9LE59	100%	100%
JS36)			(673/673)	(673/673)
At5g47780 (JS36-like)	NP_568688	Q93ZX7	63%	81%
			(290/458)	(374/458)
At2g46480	NP_182171		61%	75%
			(297/485)	(365/485)
At4g38270	NP_195540		55%	73%
A 10 0 0 10 10			(344/620)	(459/620)
At3g25140	NP_189150	Q9LSG3	53%	72%
(Quasimodo)			(241/450)	(330/450)
At1g18580	AAK93644		48%	67%
A40			(226/469)	(317/469)
At3g02350	NP_566170	Q9FWA4	47%	66%
440-00040			(247/521)	(350/521)
At2g20810	NP_565485	Q93VL7	46%	68%
444 00700			(215/462)	(320/462)
At1g06780	NP_563771	Q9M9Y5	44%	63%
A4000575	115 555		(204/461)	(296/461)
At2g30575	NP_850150		43%	65%
A40-04040	112 122		(203/463)	(309/463)
At3g01040	NP_186753	Q9MAB8	42%	61%
A45-45470	110 (000)		(189/447)	(227/447)
At5g15470	NP_197051	Q9LF35	42%	61%
A4E-E4600	ND 00000	+	(189/443)	(274/443)
At5g54690	NP_200280	Q9FH36	38%	60%
A42-29650 ( 1622)	ND FOEDOS	+	(169/436)	(265/436)
At2g38650 (JS33)	NP_565893	Q949N9	36%	60%
At3g58790	ND 404400		(171/475)	(286/475)
At3936790	NP_191438	Q9LXS3	34%	52%
GalAT-Like Family		-	(160/458)	(247/458)
At1g02720	ND 474770			
At 1902720	NP_171772	}	26	44
At1g13250	ND FC200F	OOFW74	(85/316)	(143/316)
At 19 10200	NP_563925	Q9FX71	23	41
At1g19300	NP_564077	OOI NICO	(86/359)	(154/359)
At 19 19300	NF_304077	Q9LN68	29	49
At1g24170	NP_173827	040604	(58/198)	(98/198)
7.1192-170	NF_1/302/	O48684	23	41
At1g70090	NP_564983	004536	(75/322)	(136/322)
	141-704900	004556	27	48
At3g06260	NP_187277	Q9M8J2	(64/233)	(115/233)
	111 _ 101211	GSIVIOUZ	29 (52/170)	51
At3g28340	NP_189474	Q9LHD2	(52/179) 28	(92/179)
3-00 .0	1007/7	GOLITUZ		52
At3g50760	NP_190645	Q9S7G2	(56/194) 24	(104/194)
3-0-00	100040	400762	(76/308)	43
At3g62660	NP_191825	Q9LZJ9	29	(137/308)
<b>3</b>		GOLLOS	(56/191)	51
At4g02130	NP_192122	<del>                                     </del>	29	(99/191) 51
			(58/ <u>1</u> 97)	
<del></del>		<del></del>	(30/19/)	(103/197)

The expression of the GALAT1 gene in transiently transfected mammalian cells as demonstrated herein now allows the production of stably transformed cell lines that produce GALAT1 and experiments aimed at characterizing the mechanism of the enzyme and at determining the role of GalAT1 in pectin synthesis. Specifically, the substrate specificity of GalAT1 will indicate whether it catalyzes only HGA synthesis, or also plays a role in RG-I and RG-II synthesis. Characterization of the kinetics of GalAT1 can clarify whether or not UDP-GalA is both a substrate and an allosteric regulator of the enzyme. Characterization of the mutated GalA1 enzyme can provide information regarding amino acids important in catalysis and substrate binding. The subcellular location of GALAT1 will provide the first framework for where, within the Golgi and plant endomembrane system the complex series of pectin biosynthetic reactions occur. The invention can further be used to generate transgenic plants with modified pectin, which can provide information regarding the role of GALAT1 in pectin synthesis, provide novel biosynthesis acceptors, and provide information about the role of pectin in plant growth and development. This biosynthesis framework allows further identification of GALAT1 binding proteins that would be putative pectin biosynthesis complex members. The results of these studies can serve as the foundation for a full in vitro reconstitution of functional pectin synthesis complexes.

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GALAT1 has high sequence similarity to 14 other *Arabidopsis* proteins as shown in Table IV and to proteins expressed in other plants. Possible *GALAT1* homologs in other plants are a 68 kd protein expressed in *Cicer arietinum* (chickpea) epicotyls (76% amino acid identity; 87% similarity), a hypothetical protein from *Oryza sativa* (japonica) (59% identify; 75% similarity) and a protein from *Populus alba* (49% identity; 72% similarity). Thus, the results from the study of GALAT1 in *Arabidopsis* can be extended to other plants, including those of high agricultural value.

## Heterologous expression of GALAT1

As described above, the media from human embryonic kidney (HEK293) cells transiently infected with recombinant expression vector bearing truncated *GALAT1* expressed GALAT1. Whereas transient expression allowed the

expression of sufficient GALAT to measure GalAT activity, additional expression strategies can be readily devised to produce large quantities of GALAT1 required for further characterization of the enzyme and for antibody production. Since the transiently expressed N-terminal epitope-tagged GALAT1 expressed in mammalian cells was active, one strategy is to produce stably transfected clonal HEK293 lines<sup>75</sup> expressing the same protein. The alternative strategy is to express the full length and N-terminal truncated forms of GALAT1 in the fungal expression system *Pichia pastoris*. These systems were chosen since we and others<sup>56-58</sup> have successfully used them to express plant glycosyltransferases.

For expression in *P. pastoris*, cDNA encoding the entire, and the truncated soluble forms of GALAT can be generated by PCR using gene/vector specific primers. The PCR products are then subcloned into appropriate *Pichia* expression vectors (Invitrogen, Carlsbad, CA) in which the cDNA is inserted downstream from an alcohol oxidase (AOX1) promoter. We have made full length coding sequence constructs for expression in the *Pichia* vector pPIC 3.5. This vector does not contain an epitope tag. One can easily make epitope tagged GALAT1 constructs in the *Pichia* vectors pPICz and pPICzα (Invitrogen) and determine whether functional C-terminal epitope-tagged constructs that do not affect GalAT activity can be recovered. Several studies have demonstrated success of the *Pichia* system<sup>76-82</sup>. Once a high-GALAT1-producing line is recovered, production of large amounts of protein can be carried out in fermentors or spinner flasks.

# Characterization of Expressed GALAT1

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To begin to address how HGA is synthesized, the kinetics, substrate specificity, and structure of the purified recombinantly expressed GALAT1 can be determined and compared to the solubilized membrane-bound *Arabidopsis* GALAT purified by immunoadsorption using the polyclonal-antiGALAT1 (see below). Although the characteristics of GalAT1 are consistent with the enzyme being the/a catalytic subunit of the HGA synthase, GALAT1 could be a GalAT involved in RG-II or RG-I synthesis. For example, GalAT could represent an RG-I:GalAT that initially elongates HGA by a single GalA and then waits for a required NDP-Rha to start RG-I backbone synthesis. The kinetics of purified and recombinantly expressed GALAT1 for UDP-GalA and a size range of homogalacturonan and pectin

acceptors can be determined. The effect of other nucleotide-sugars and oligosaccharide substrates on GalAT can also be tested to identify activators and inhibitors.

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The expressed full length and truncated enzymes can be assayed in a reaction buffer in the presence, and absence, respectively, of Triton X-100. The kinetics of the enzyme for UDP-GalA can be carried out in a total of 1 µM to 80 mM UDP-GalA + UDP-[14C]GalA. We routinely synthesize UDP-[14C]GalA either by the 4epimerization of UDP-[14C]GlcA1 or oxidation of UDP-[14C]Gal84 since UDP-[14C]GalA is not commercially available. The effect of different acceptors on GALAT1 activity can be conducted using 100 µM UDP-GalA and 0.1-100 µg acceptor/ 30 µl reaction. The acceptors to be tested include HGA oligosaccharides (oligogalacturonides) of degrees of polymerization ranging from 2-16, polygalacturonic acid, commercially available citrus pectin of ~30, 60 and 90% esterification, RG-I and RG-II. The products made using the different acceptors can be characterized<sup>2,3</sup>. If RG-I is shown to serve as an acceptor, RG-I backbone fragments that have a GalA or a Rha at the non-reducing end can be used to determine acceptor specificity. The acceptors can be tested using multiple assays including the precipitation assay<sup>2</sup> and a filter assay<sup>63</sup>. The enzymes can also be tested for the effect of pH, temperature, reducing agents, divalent cations and salts on enzyme activity and product structure.

Characteristics of the recombinant truncated GALAT1 can be compared to the GALAT1 solubilized from *Arabidopsis* membranes by immunoadsorption of the solubilized GALAT1 using anti-GALAT1 antibody (see section below) bound to Protein A or G Sepharose, or by coupling the anti-GALAT1 antibodies to 3M-Emphaze resin<sup>86</sup> and using the resin used to purify GALAT1 from solubilized *Arabidopsis* enzyme. If the characteristics of the immunoadsorbed *Arabidopsis* GALAT1 are different from those of the recombinant truncated GALAT1, the immunoadsorbed GALAT1 can be analyzed by LC tandem mass spectrometry to determine if additional proteins are immunoadsorbed with the *Arabidopsis* solubilized GALAT1 that may have modified the activity (e.g. a heteromeric complex).

The recombinant GALAT1 and the GALAT1 immunoadsorbed-from Arabidopsis solubilized membranes can also be treated with N-glycanase to determine if they are N-glycosylated. To determine if they are O-glycosylated, the proteins be exhaustively treated with N-glycanase, the released can oligosaccharides removed, and the resulting protein analyzed by TMS methylation analysis to determine the glycosyl residue composition of any carbohydrates still Any oligosaccharide released by the N-glycanase attached to the protein. treatment can also be analyzed by TMS methylation. The results of these experiments would indicate whether the native Arabidopsis GalAT is glycosylated and whether the recombinant forms have the same or different glycosylation Changes in glycosylation could affect GalAT1 enzyme activity and/or substrate binding. GALAT1 is predicted to have 5 or 6 N-glycosylation sites (NetNGlyc 1.0 Prediction; <a href="http://www.expasy.org/sitemap.html">http://www.expasy.org/sitemap.html</a>).

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As mentioned above, we have found that membrane-bound and solubilized GalAT activity in tobacco and radish has unusual apparent biphasic kinetics. Thus, we are particularly interested in determining if the expressed GALAT1 shows the same kinetics, including possible allosteric regulation by UDP-GalA. One can test for possible multimeric structure by determining the mass of the enzyme by size exclusion chromatography and comparing these with the mass obtained by SDS-PAGE. The possibility that GALAT1 exists as a heteromultimer can be tested by mixing expressed recombinant GALAT1 with solubilized *Arabidopsis* enzymes and immunoadsorbing GALAT1 and proteins bound to it using either an anti-GALAT1 antibody or an anti-HA epitope antibody (see previous section).

# Production of a series of mutated GALAT1 proteins by site-directed mutagenesis

As discussed above, there are 45 conserved amino acids in GALAT1 among the 15 members of the *GALAT* family. To determine the role of these residues in substrate/acceptor binding and/or catalysis, each amino acid is systematically mutated using site-directed mutagenesis. The effect of these mutations on GALAT1 specific activity, and where warranted, on Km, Vmax, and acceptor specificity (i.e. OGA, RG-I and RG-II) and product size (i.e. enzyme processivity) is determined.

## Production and use of antibodies

antibodies are necessary for the immunocytochemistry Anti-GalAT experiments, to immunopurify solubilized GALAT1 from Arabidopsis, and to select proteins that potentially bind to GALAT1 and may function in pectin biosynthetic enzyme complexes. A skilled artisan can generate anti-GalAT antibodies using the nucleic acid or amino acid sequences disclosed herein. This can be accomplished by employing the heterologously expressed truncated or full-length GALAT1. Alternatively, a small peptide derived from the GALAT1 sequence can be synthesized and used to generate anti-GALAT1 antibodies. One can generate either polyclonal or monoclonal antibodies. Such antibodies are useful for a range of experiments, including subcellular immunocytochemistry, immunoprecipitation/adsorption, and enzyme activity inhibition studies. Monoclonal or polyclonal antibodies, specifically reacting with a protein of interest can be made by methods well known in the art. See, e.g., Harlow and Lane (1988) Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratories; Goding (1996) Monoclonal Antibodies: Principles and Practice, 3rd ed., Academic Press, San Diego, CA, and Ausubel et al. (1993) Current Protocols in Molecular Biology, Wiley Interscience/Greene Publishing, New York, NY.

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## Subcellular localization of GALAT1

All available data, including the localization of the catalytic domain of GalAT in the Golgi lumen<sup>7</sup>, suggest that pectin is synthesized in the Golgi and transferred via vesicles to the wall. However, it is not known how the different glycosyltransferases function to make specific pectin structures. We predict that different glycosyltransferases are localized in a sequential manner to different cisternae of the Golgi<sup>22,91</sup> in an order indicative of the order in which pectin is synthesized as it moves from the cis, through the medial and to the trans Golgi. Evidence from both animal<sup>92,93</sup> and plants<sup>94</sup> suggests that, either individually or in combination, the transmembrane domain (i.e. the bilayer thickness model<sup>95</sup>), the N-or C-terminal sequences flanking the transmembrane domain, and/or the lumenal domain (i.e. the 'kin recognition model'<sup>96</sup>) contribute to localization of proteins within the Golgi system. The anti-GalAT antibodies generated as described above can be used to determine the subcellular localization of GALAT1 within the Golgi in order

to provide additional information on the role of GalAT1 in pectin synthesis. For example, a location of GALAT1 in the cis and medial Golgi cisternae would be consistent with a function of GALAT1 in HGA synthesis, while a localization primarily in the late medial or trans Golgi would be more suggestive of a role in RG-I or possible RG-II synthesis. It should be noted that such subcompartment localization studies, while important and novel for the pectin biosynthetic enzymes, are also novel in any species since the "precise location of only a small number of the glycosyltransferase proteins within the Golgi apparatus have been determined" Anti-GALAT1 antibodies can be used to identify where in the Golgi GALAT1 is localized by, for example, immunogold label of thin sections from Arabidopsis P1, 91,98, 99 including both developing Arabidopsis seedlings and growing suspension cultures which have cells actively making wall.

# Use of mutants and RNAi to generate and characterize *GALAT1* and GalAT gene Superfamily knockouts.

Double-stranded RNA-mediated interference (RNAi) is a method to study the function of genes in plants <sup>100</sup>. Transgenic plants harboring an RNAi construct often have reduced expression of the gene-specific mRNA. The resulting plants may display either complete gene silencing, thus having a knockout phenotype, or a partial "knockout" phenotype due to 'leaky' expression. The RNAi approach should allow the suppression of *GALAT1* expression and a reduction or loss of GALAT1. This enables one to elucidate the function of GALAT in pectin synthesis and in the plant. Simultaneously, the sequence-indexed T-DNA insertion mutants listed in the Salk Institute Genomic Analysis Laboratory (SIGnAL) *Arabidopsis* T-DNA mutant collection (<a href="http://signal.salk.edu/cgi-bin/tdnaexpress">http://signal.salk.edu/cgi-bin/tdnaexpress</a>) can be monitored to determine if any T-DNA insert lines for GALAT become available. If so, the seed can be obtained and the mutants generated therefrom can be characterized (as described above).

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The putative pectin biosynthesis mutants can aid in the identification of gene function in two ways. The visible phenotypes of the mutants can provide information on the biological function of the gene (if there is no redundancy in gene function) by demonstrating when during growth and development the particular

gene product is needed (as shown above). Structural analysis of the pectin in the mutant walls can provide information about the specific enzyme activity of the gene in pectin synthesis (as shown above).

Of particular importance regarding pectin synthesis, the cell walls are isolated and analyzed for glycosyl residue composition (see above) and linkage to provide information about the possible role of GALAT1 in pectin synthesis.

# Identification of the members of HGA biosynthetic complexes.

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There is growing evidence that glycoconjugates are synthesized by complexes of glycosyltransferases and other types of proteins<sup>102</sup>. For example, ganglioside synthesis occurs via a tightly regulated formation of multiple glycosyltransferase complexes<sup>102</sup>. Thus, any protein members of HGA biosynthetic complexes can be isolated by immunoadsorbing such proteins bound to GALAT1 using anti-GALAT1 antibodies or anti-HA epitope antibodies. The immunoadsorbed proteins can be identified by SDS-PAGE, removed from the gel, and their amino acid sequence determined by LC-tandem mass spectrometry. The amino acid sequences thus obtained can then be used to search the available protein databases for their identities.

# Characterization of mutant phenotypes and bulking up of seed.

A person of ordinary skill in the art can use mutant seeds to probe gene function. For example, the initial mutant seed (often a segregating T3 line, see http://signal.salk.edu/tdna\_FAQs.html) can be grown and selfed to increase the seed stock (T4). Multiple plants from T4 seed can be grown and the presence of, for example) the T-DNA insert determined by PCR of plant genomic DNA using a T-DNA primer and a gene specific primer. The same DNA can be analyzed with gene specific primers that should span the T-DNA insertion site. These analyses should indicate whether the given plant contains a T-DNA insert and if so, whether it is homozygous or heterozygous for the mutation. If necessary, Southern blotting and hybridization with the specific genes can be used to determine if the gene contains the expected T-DNA insert. Seed homozygous for the T-DNA insertion (when not lethal) or heterozygous (when no viable TDNA homozygous plants are obtained) can be selfed to amplify the seed and, for heterozygous plants, to test for

segregation of any phenotype or T-DNA insert. Plants can be scored as heterozygous or homozygous by PCR analysis of the T-DNA insert and by any visible phenotype. Homozygous or heterozygous plants can be used for growth phenotype and cell wall analysis. The seed can also be crossed with wild type Columbia and then selfed to eliminate the possibility that the lines contain an unexpected mutation or additional T-DNA insert(s).

#### **Growth Phenotype analysis**

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Several growth parameters of the mutant and wild type plants are recorded to yield a general phenotypic characterization of the mutant plants. 134

#### Analysis of Cell Walls

Homozygous or heterozygous plants are grown and analyzed for wall composition and linkage. Cell walls can, for example, be prepared as alcohol insoluble residues (AIRs) from WT and (homozygous) mutant *Arabidopsis* plant tissues<sup>135</sup>. AIRs are prepared by homogenizing leaves and stems (from soil-grown plants) and roots (from liquid-cultured plants) in aquous 80% EtOH followed by washes with absolute EtOH, chloroform-methanol, and acetone. Separate fractions containing RG-I, RG-II and oligogalacturonides can be obtained by size-exclusion chromatography (SEC) and ion exchange chromatography of the material solubilized from the cell walls by treatment with pectin methyl esterase (PME) and *endo*-polygalacturonase (EPG). The yields, glycosyl residue compositions, and glycosyl linkage compositions of each fraction can be determined<sup>27</sup>.

The nucleotide and amino acid sequences of the fifteen *GALAT* gene family members are shown as follows.

### Sequence #1 (SEQ ID NO:1)

45

Gene name: At3g61130
GeneBank accession # for reference: NM\_115977 GI:18411855
Nucleotide sequence of Sequence #1:
Positions 1-2022 of CDS of NM\_115977.

10 1 atggcgctaa agcgagggct atctggagtt aaccggatta gaggaagtgg tggtggatct 61 cgatctgtgc ttgtgcttct catatttttc tgtgtttttg cacctctttg cttctttgtt 121 ggccgaggag tgtatatcga ttcctcaaat gattattcaa ttgtttctgt gaagcagaat 181 cttgactgga gagaacgttt agcaatgcaa tctgttagat ctcttttctc gaaagagata 241 ctagatgtta tagcaaccag cacagctgat ttgggtcctc ttagccttga ttcttttaag 301 aaaaacaatt tgtctgcatc atggcgggga accggagtag acccctcctt tagacattct 15 361 gagaatecag caacteetga tgteaaatet aataacetga atgaaaaaeg tgacageatt 421 tcaaaagata gtatccatca gaaagttgag acacctacaa agattcacag aaggcaacta 481 agagagaaaa ggcgtgagat gcgggcaaat gagttagttc agcacaatga tgacacgatt 541 ttgaaactcg aaaatgctgc cattgaacgc tctaagtctg ttgattctgc agtccttggt 601 aaatacagta tttggagaag agaaaatgag aatgacaact ctgattcaaa tatacgcttg 20 661 atgcgggatc aagtaataat ggctagagtc tatagtggga ttgcaaaatt gaaaaacaag 721 aacgatttgt tacaagaact ccaggcccga cttaaggaca gccaacgggt tttgggggaa 781 gcaacatctg atgetgatct teeteggagt gegeatgaga aacteagage catgggteaa 841 gtcttggcta aagctaagat gcagttatat gactgcaagc tggttactgg aaagctgaga 25 901 gcaatgcttc agactgccga cgaacaagtg aggagcttaa agaagcagag tacttttctg 961 gctcagttag cagcaaaaac cattccaaat cctatccatt gcctatcaat gcgcttgact 1021 atcgattact atcttctgtc tccggagaaa agaaaattcc ctcggagtga aaacctagaa 1081 aaccctaatc tttatcatta tgccctcttt tccgacaatg tattagctgc atcagtagtt 1141 gttaactcaa ccatcatgaa tgccaaggat ccttctaagc atgtttttca ccttgtcacg 1201 gataaactca atttcggagc aatgaacatg tggttcctcc taaacccacc cggaaaggca 30 1321 cttcgtcagc ttgaatctgc agcaatgaga gagtactatt ttaaagcaga ccatccaact 1381 tcaggctctt cgaatctaaa atacagaaac ccaaagtatc tatccatgtt gaatcacttg 1441 agattetace teeetgaggt ttateceaag etgaacaaaa teetetteet ggacgatgae 1501 atcattgttc agaaagactt gactccactc tgggaagtta acctgaacgg caaagtcaac 35 1561 ggtgcagtcg aaacctgtgg ggaaagtttc cacagattcg acaagtatct caacttttcg 1621 aatcctcaca ttgcgaggaa cttcaatcca aatgcttgtg gatgggctta tggaatgaac 1681 atgttcgacc taaaggaatg gaagaagaga gacatcactg gtatatacca caagtggcaa 1741 aacatgaatg agaacaggac actatggaag ctagggacat tgccaccagg attaataaca 1801 ttctacggat taacacatcc cttaaacaag gcgtggcatg tgctgggact tggatataac 40 1861 ccgagtatcg acaagaagga cattgagaat gcagcagtgg ttcactataa cgggaacatg 1921 aaaccatggt tggagttggc aatgtccaaa tatcggccgt attggaccaa gtacatcaag 1981 tttgatcacc catatetteg tegttgeaac etteatgaat aa

Amino Acid Sequence of Sequence #1: (SEQ ID NO:2) GeneBank ID# NP\_191672 Positions 1-673 of NP\_191672.

5

1 malkrglsgv nrirgsgggs rsvlvlliff cvfaplcffv grgvyidssn dysivsvkqn
61 ldwrerlamq svrslfskei ldviatstad lgplsldsfk knnlsaswrg tgvdpsfrhs
121 enpatpdvks nnlnekrdsi skdsihqkve tptkihrrql rekrremran elvqhnddti
181 lklenaaier sksvdsavlg kysiwrrene ndnsdsnirl mrdqvimarv ysgiaklknk
10 241 ndllqelqar lkdsqrvlge atsdadlprs aheklramgq vlakakmqly dcklvtgklr
301 amlqtadeqv rslkkqstfl aqlaaktipn pihclsmrlt idyyllspek rkfprsenle
361 npnlyhyalf sdnvlaasvv vnstimnakd pskhvfhlvt dklnfgamnm wfllnppgka
421 tihvenvdef kwlnssycpv lrqlesaamr eyyfkadhpt sgssnlkyrn pkylsmlnhl
481 rfylpevypk lnkilflddd iivqkdltpl wevnlngkvn gavetcgesf hrfdkylnfs
541 nphiarnfnp nacgwaygmn mfdlkewkkr ditgiyhkwq nmnenrtlwk lgtlppglit
601 fyglthplnk awhvlglgyn psidkkdien aavvhyngnm kpwlelamsk yrpywtkyik
661 fdhpylrrcn lhe

#### 20 Sequence #2 (SEQ ID NO:3)

Gene name: At2g38650

GeneBank accession # for reference: NM 129422 GI:30687590

Nucleotide sequence of Sequence #2:

25 Positions 1-1860 of CDS of NM 129422

1 atgaaaggcg gaggcggtgg tggaggaggt ggtggcggag gaaaacgccg gtggaaagtt 61 ctggtgattg gagttttggt tcttgttatt ctttctatgc ttgttcctct tgctttctta 121 ctcggtcttc acaatggctt tcactctcct ggatttgtca ctgttcaacc ggcttcttca 30 181 tttgagaget ttaccagaat caatgetact aageatacae agagagatgt atccgaacgg 241 gtcgatgagg ttcttcaaaa aatcaatcca gttcttccca agaaaagcga cataaacgtg 301 ggttccagag atgtgaatgc aacaagcggc actgattcta aaaaaagagg attaccagtg 361 tecceaactg ttgttgecaa tecaageeet geaaataaaa caaaategga ageeteatat 421 acaggtgttc agaggaaaat agtaagtggt gatgaaactt ggagaacttg tgaagtgaaa 481 tatgggaget actgcctctg gagggaggaa aataaggaac caatgaaaga tgccaaggtg 35 541 aagcaaatga aggaccagct gtttgtggct agagcatact atcccagtat tgctaaaatg 601 ccttctcaaa gcaagttgac tcgggatatg aaacagaata tccaagagtt tgagcgtatt 661 cttagtgaaa gttctcaaga tgctgacctt ccaccacagg ttgataaaaa gttgcagaag 721 atggaagetg taattgcaaa ggcaaagtet tttccagteg actgtaacaa tgttgacaag 40 781 aaattgagac agatcettga tttgactgag gatgaageta gtttccacat gaaacagagt 841 gtgttcctct accagcttgc agtacagaca atgcctaaga gtcttcattg cttgtcaatg 901 cgactaactg tggaacattt caagtcagat tcacttgagg atcccattag tgagaaattt 961 tcagatccct cattacttca ctttgttatc atctccgata atatactagc atcgtccgtt 1021 gtgatcaact caacggttgt acatgcaagg gacagtaaaa actttgtttt ccatgtactg 1081 acagacgage agaattactt tgcaatgaaa caatggttta ttaggaatee ttgcaaacaa 45 1141 tcaactgttc aagtattgaa cattgaaaaa ctcgagctgg acgattctga tatgaaactg 1201 tetttgtetg eggagtteeg tgttteette eeeagtggtg acettttgge gteteaacag 1261 aatagaacac actacttatc ccttttctct caatctcact atcttcttcc caaattattt 1321 gacaaattgg agaaggttgt gattctggat gatgacgttg tagtccagcg agacttatct 50 1381 cccctttggg accttgatat ggaagggaaa gtgaatggcg ctgttaagtc gtgcactgtg

1441 agattgggtc agctaaggag tctcaagaga ggaaattttg ataccaatgc ttgtctctgg
1501 atgtctggtt tgaatgtcgt tgatcttgct agatggaggg cattgggtgt ttcagaaacc
1561 tatcaaaaat attataaaga gatgagtagt ggagatgagt cgagcgaagc aattgcattg
1621 caggcaagct tgctcacatt tcaagaccaa gtatatgctc ttgacgacaa atgggctcta
1681 tcagggcttg gttatgacta ctacatcaat gcacaagcca taaaaaacgc agccatattg
1741 cactataacg ggaacatgaa gccgtggctt gagctgggaa tcccaaatta caaaaactat
1801 tggagaaggc atctgagtcg ggaagatcgg ttcttgagtg actgtaacgt gaatccttga

- Amino Acid Sequence of Sequence #2: (SEQ ID NO:4)
  GeneBank ID# NP\_565893
  Positions 1-619 of NP 565893.
- 1 mkggggggg ggggkrrwkv lvigvlvlvi lsmlvplafl lglhngfhsp gfvtvqpass
  61 fesftrinat khtqrdvser vdevlqkinp vlpkksdinv gsrdvnatsg tdskkrglpv
  121 sptvvanpsp anktkseasy tgvqrkivsg detwrtcevk ygsyclwree nkepmkdakv
  181 kqmkdqlfva rayypsiakm psqskltrdm kqniqeferi lsessqdadl ppqvdkklqk
  241 meaviakaks fpvdcnnvdk klrqildlte deasfhmkqs vflyqlavqt mpkslhclsm
  301 rltvehfksd sledpisekf sdpsllhfvi isdnilassv vinstvvhar dsknfvfhvl
  20 361 tdeqnyfamk qwfirnpckq stvqvlniek lelddsdmkl slsaefrvsf psgdllasqq
  421 nrthylslfs qshyllpklf dklekvvild ddvvvqrdls plwdldmegk vngavksctv
  481 rlgqlrslkr gnfdtnaclw msglnvvdla rwralgvset yqkyykemss gdesseaial
  541 qaslltfqdq vyalddkwal sglgydyyin aqaiknaail hyngnmkpwl elgipnykny
  601 wrrhlsredr flsdcnvnp

#### Sequence #3 (SEQ ID NO:5)

Gene name: At5g47780

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GeneBank accession # for reference: NM\_124152 GI:30695292

Nucleotide sequence of Sequence #3: Positions 1-1851 of CDS of NM\_124152.

1 atgatggtga agettegeaa tettgttett ttetteatge teeteacegt cottgeteat 61 atcettetet acacegatee egetgeetee tteaagacee cettttetaa acgegattte 35 121 ctcgaggacg taaccgcctt gactttcaat tccgatgaga atcgtttgaa tcttcttcct 181 cgggaatete eegetgtget eagaggagga etegteggtg etgtetatte egataagaat 241 tcacggcggc tagaccaatt gtctgctcga gttctttccg ccaccgacga tgatactcac 301 tcacatactg acatttccat caaacaagtc actcatgatg cagcctcaga ctcgcatatt 361 aatagggaaa atatgcatgt tcaattgacc caacaaacct ctgaaaaagt tgatgagcaa 421 ccagagccta atgcttttgg agctaagaaa gatactggaa acgtgttgat gcctgatgct 40 481 caagtgaggc atcttaaaga tcagcttatt agggcaaagg tttatctttc ccttccatct 541 gcaaaggcca atgctcattt tgtgagagag cttcgactcc gtattaaaga agttcaacgg 601 gcacttgcag atgcctccaa ggattcggat ctgccaaaga ctgctataga aaagctaaaa 661 gcaatggagc aaacactggc caaaggcaag cagatccaag atgactgttc tacagtggtc 45 721 aagaagctac gtgctatgct ccactccgca gatgagcagc tacgggtcca taagaagcaa 781 accatgtttt tgactcaatt gactgctaag accattccta aaggacttca ctgccttcct 841 ctgcgcctca ctacagacta ttatgcttta aattcatctg aacaacaatt tccaaatcag 901 gagaaactag aagatactca getgtateae tatgeeettt tetetgataa tgttttgget 961 acgtcagttg ttgttaactc taccataacc aatgcaaagc atcccttaaa gcatgtcttc 1021 cacategica cagacagact caattatgeg geaatgagga tgtggtteet ggacaateea 50

PCT/US2004/003545 WO 2004/072250

1081 cctggcaaag ccaccatcca ggttcagaat gttgaagaat ttacatggct gaattcaagc 1141 tacagteceg tteteaaaca gettagttet agategatga tagattatta etteagagee 1201 caccatacaa attcagacac caacttgaag ttccggaatc caaaatactt atcgatcctt 1261 aatcatette gtttttaett geetgagate ttteecaage teageaaagt getettettg 5 1321 gatgatgata tagttgtgca gaaggacctt tctggtcttt ggtcagttga tctgaaaggt 1381 aatgttaacg gtgctgtaga gacgtgtggg gaaagctttc atcgctttga ccgttatctg 1441 aactteteaa ateeacteat tteeaagaae tttgaceete gagettgtgg ttgggegtat 1501 ggtatgaatg tctttgatct ggatgaatgg aagaggcaaa acatcacaga agtttatcat 1561 cgatggcagg atctgaatca agaccgagaa ttgtggaagc tagggacgtt gccgcctggt 1621 ctaatcacat tttggagacg aacatatccg ctagaccgga aatggcacat actagggctt 10 1681 ggatacaacc cgagtgtgaa ccaaagggat attgagaggg cagccgtgat acactataat 1741 ggcaacctca aaccatggct agagattggg attccaagat acagaggctt ctggtcaaag 1801 catgtagact atgagcacgt ttatctcaga gaatgcaaca tcaatcctta g

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Amino Acid Sequence of Sequence #3: (SEQ ID NO:6) Genebank ID# NP 568688 Positions 1-616 of NP\_568688.

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1 mmvklrnlvl ffmlltvvah illytdpaas fktpfskrdf ledvtaltfn sdenrinlip 61 respavlrgg lvgavysdkn srrldqlsar vlsatdddth shtdisikqv thdaasdshi 121 nrenmhvqlt qqtsekvdeq pepnafgakk dtgnvlmpda qvrhlkdqli rakvylslps 181 akanahfvre Irlrikevqr aladaskdsd lpktaieklk ameqtlakgk qiqddcstvv 241 kklramlhsa deqlrvhkkq tmfltqltak tipkglhclp Irlttdyyal nsseqqfpnq 301 ekledtqlyh yalfsdnvla tsvvvnstit nakhplkhvf hivtdrlnya amrmwfldnp 361 pakatiqvan veeftwinss yspvlkalss rsmidyyfra hhtnsdtnik frnpkylsil 421 nhlrfylpei fpklskvlfl dddivvqkdl sglwsvdlkg nvngavetcg esfhrfdryl 481 nfsnpliskn fdpracgway gmnvfdldew krqnitevyh rwqdlnqdre lwklgtlppg 541 litfwrrtyp Idrkwhilgl gynpsvnqrd ieraavihyn gnlkpwleig ipryrgfwsk 601 hvdyehvylr ecninp

## Sequence #4 (SEQ ID NO:7)

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Gene name: At1g06780 GeneBank accession # for reference: NM\_100555 GI:30679825 Nucleotide sequence of Sequence #4: Positions 1-1770 of CDS of NM 100555.

40

1 atgaaacaaa ttcgtcgatg gcagaggatt ttgatcctcg ctctgctatc gatatcagta 61 ttcgctccgc ttattttcgt atcgaatcgg cttaagagca tcactcccgt tggtcgtaga 121 gaatttattg aagagttatc caaaattaga ttcacgacaa atgaccttag acttagcgct 181 attgaacatg aggatggaga aggcttgaag gggccaaggc tcattctctt caaggatggg 241 gagtttaatt cgtctgctga aagtgatggt ggtaatactt acaaaaacag ggaagaacaa 45 301 gtgattgttt cacagaagat gacagttagc tctgatgaaa agggtcaaat tctaccaaca 361 gtcaaccaac ttgctaataa aacggatttc aagccccctt tatctaaggg tgaaaagaac 421 acaagggttc agcccgacag agcaacagat gtgaaaacga aggagatcag agacaaaatt 481 attcaagcta aagcctacct gaatttcgct ccacctggaa gtaactctca agttgtgaag 541 gagttgagag gtcggctgaa agagctggaa cggtctgttg gtgatgcaac aaaggacaag 50 601 gacttatcaa agggcgctct ccgcagggtg aagcccatgg aaaatgtgtt atataaggct

PCT/US2004/003545 WO 2004/072250

661 agtogtgtct ttaacaattg coctgccatc gctaccaaac tccgtgccat gaattataac 721 acagaagaac aagttcaggc gcagaaaaat caagcagcgt atctaatgca gcttgcagca 781 aggaccaccc caaaagggct tcactgtctc tcaatgcggc tgacatcaga atacttttca 841 ctggatcctg aaaaaaggca gatgcctaac cagcaaaatt attttgacgc taatttcaat 901 cattatgttg tcttctctga caatgttttg gcttcttcag tcgttgttaa ctctacgata 5 961 tetteateaa aggageeaga aagaatagte tteeatgteg tgaetgatte aettaattae 1021 ccagcaatct caatgtggtt tctgctaaac attcaaagta aagctactat ccaaatccta 1081 aacattgatg atatggatgt cctgcctaga gattatgatc aattactgat gaagcaaaac 1141 tctaatgacc caagattcat ttctacactc aatcacgcac gcttctatct cccggatata 10 1201 ttcccgggtt tgaacaagat ggtactcttg gaccatgatg tagttgttca aagagattta 1261 agtagactgt ggagcattga tatgaaagga aaggtggttg gagctgtaga gacttgtctt 1321 gaaggtgaat cttcatttcg atcaatgagc acatttatta atttctcaga cacatgggtc 1381 gctgggaaat ttagtcctag agcttgcaca tgggctttcg ggatgaatct aattgatctc 1441 gaagaatgga gaatacggaa gttgacttct acatacataa aatacttcaa cctgggaaca 1501 aagagaccat tgtggaaagc tgggagctta ccaataggtt ggttgacttt ctataggcaa 15 1561 acattagcat tggacaagag atggcatgtg atggggttag gtcgcgaatc aggagtcaaa 1621 geggttgaca tegaacaage ggeagttata cactaegatg gggteatgaa geegtggttg 1681 gacattggaa aagagaatta caaacgttac tggaacatac acgtccctta ccatcacacc 1741 tacttgcaac agtgcaatct tcaagcttga

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Amino Acid Sequence of Sequence #4: (SEQ ID NO: 8) Genebank ID# NP 563771 Positions 1-589.

25

1 mkgirrwgri lilallsisv faplifysnr lksitpygrr efieelskir fttndlrlsa 61 iehedgeglk gprlilfkdg efnssaesdg gntyknreeq vivsqkmtvs sdekgqilpt 121 vnqlanktdf kpplskgekn trvqpdratd vktkeirdki iqakaylnfa ppgsnsqvvk 181 elrgrikele rsvgdatkdk diskgalrrv kpmenvlyka srvfnncpai atkiramnyn 30 241 teeqvqaqkn qaaylmqlaa rttpkglhcl smrltseyfs idpekrqmpn qqnyfdanfn 301 hyvvfsdnvl assvvvnsti ssskeperiv fhvvtdslny paismwflln iqskatiqil 361 niddmdvlpr dydqllmkqn sndprfistl nharfylpdi fpglnkmvll dhdvvvqrdl 421 srlwsidmkg kvvgavetcl egessfrsms tfinfsdtwv agkfspract wafgmnlidl 481 eewrirklts tyikyfnlgt krplwkagsl pigwltfyrq tlaldkrwhv mglgresgvk 541 avdieqaavi hydgvmkpwl digkenykry wnihvpyhht ylqqcnlqa

### Sequence #5 (SEQ ID NO:9)

Gene name: At1g18580

GeneBank accession # for reference: AY062444 GI:17064735

Nucleotide sequence of Sequence #5: Positions 1-1614 of CDS of AY062444.

1 atgaggcggt ggccggtgga tcaccggcgg cgaggtagaa ggagattgtc gagttggata 10 61 tggtttctcc ttggttcttt ctctgtcgct ggtttagttc tcttcatcgt tcagcattat 121 caccatcaac aagatccatc ccagctttta cttgagagag acacgagaac cgaaatggta 181 tetectecce atttaaaett caeggaagag gteacaagtg ettecteett etetaggeag 241 ttagcagagc aaatgacact tgccaaagct tatgtgttta tagctaaaga gcataataat 301 cttcatttag cttgggaatt gagttctaag atcagaagtt gtcagctttt gctttccaaa 361 gcagctatga gaggacaacc tatttcgttt gatgaggcta aaccgattat tactggtcta 15 421 tcagctctta tctacaaggc tcaagatgca cattatgata ttgccaccac tatgatgacc 481 atgaaatctc acatccaagc acttgaagag cgtgcaaatg cagctactgt tcagaccaca 541 atatttgggc aattggttgc tgaggcatta ccaaagagcc tccactgttt gacgataaag 601 ctcacatctg attgggtaac agagccatct cgccatgaac tggcagatga gaacagaaac 20 661 tcacctagac ttgtcgacaa caacctctac cacttctgca tcttctcgga caacgtgatt 721 gccacctcgg ttgttgttaa ttcaactgtc tcgaatgctg atcatccaaa gcagcttgtt 781 ttccacatag tgacgaatcg agtgagctac aaagctatgc aggcctggtt tctaagtaat 841 gacttcaagg gctcagcaat agagatcagg agcgtagagg agttttcttg gttgaatgct 901 tcatattctc ctgttgttaa gcaactgctg gacacagatg caagagctta ctatttcggg 961 gaacagacaa gtcaagatac gatttccgag ccaaaagtga ggaacccaaa gtacttgtca 25 1021 ttactgaacc atctcagatt ctacattccg gagatctatc cacagctaga gaagattgtt 1081 ttcctagacg atgatgttgt tgttcagaaa gatttgactc cactcttctc cttggatctg 1141 catggaaacg tcaatggagc tgtggaaaca tgtcttgaag cctttcaccg atattacaag 1201 tatetaaatt tetegaacee acteateage teaaagtteg acceacaage atgtggatgg 1261 gcttttggta tgaacgtttt tgatctgatc gcttggagga atgcaaacgt gactgctcgg 30 1321 taccattact ggcaagatca gaacagagaa cgaacgettt ggaaactegg gacacteect 1381 ccaggtctac tatctttcta tggtctcaca gagccactgg acagaagatg gcatgtcttg 1441 ggtttaggtt acgatgtgaa catcgataac cgtctgatcg aaacagcagc tgtgattcac 1501 tataatggta acatgaagcc ttggctaaag ctggctattg gtaggtataa acctttctgg 1561 ttaaagtttt tgaactcgag ccatccttat ttacaagatt gtgtcacagc ttaa 35

Amino Acid Sequence of Sequence #5: (SEQ ID NO: 10) Genebank ID# AAK93644 GI:15293067 Positions 1-537 of AAK93644.

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1 mrrwpvdhrr rgrrrlsswi wfllgsfsva glvlfivqhy hhqqdpsqll lerdtrtemv
61 spphlnftee vtsassfsrq laeqmtlaka yvfiakehnn lhlawelssk irscqlllsk
121 aamrgqpisf deakpiitgl saliykaqda hydiattmmt mkshiqalee ranaatvqtt
181 ifgqlvaeal pkslhcltik Itsdwvteps rheladenrn sprlvdnnly hfcifsdnvi
241 atsvvvnstv snadhpkqlv fhivtnrvsy kamqawflsn dfkgsaieir sveefswlna
301 syspvvkqll dtdarayyfg eqtsqdtise pkvrnpkyls llnhlrfyip eiypqlekiv
361 fldddvvvqk dltplfsldl hgnvngavet cleafhryyk ylnfsnplis skfdpqacgw
421 afgmnvfdli awrnanvtar yhywqdqnre rtlwklgtlp pgllsfyglt epldrrwhvl
481 glgydvnidn rlietaavih yngnmkpwlk laigrykpfw lkflnsshpy lqdcvta

## Sequence #6 (SEQ ID NO: 11)

Gene name: At2g20810

GeneBank accession # for reference: NM\_127647 GI:30681142

Nucleotide sequence of Sequence #6:Positions 1-1611 of CDS of NM 127647.

1 atgagaagga gaggaggga tagtttccgg agagctggac ggaggaagat ctcgaatgtg 61 gtatggtggg ttctctctgg tattgccctc ctgctcttct ttctcattct ctccaaagct 10 121 ggtcatattg aacctagacc ctctattcct aagcgacgtt accgtaatga caaatttgta 181 gagggtatga atatgactga ggaaatgttg agtcctactt ccgttgctcg tcaagttaat 241 gatcagattg ctcttgctaa agcttttgtt gtcattgcta aagaaagtaa gaatcttcag 301 tttgettggg acttaagtge teagateegt aacteteagt tgettttate gagtgetget 361 actaggagaa gtcccttgac tgtcttggaa tctgagtcta ctattcgtga catggctgtt 421 ttgttatatc aagctcagca gcttcactat gatagtgcta ctatgattat gaggcttaag 15 481 gcctcgattc aggctcttga agaacaaatg agttccgtta gcgagaagag ttccaagtat 541 ggacagattg ctgctgagga agtgcctaag agtctttact gtcttggtgt tcgtctcact 601 accgaatggt ttcagaattt agacttacag agaactctta aggaaaggag tcgtgttgat 661 tcgaaactca cggataacag tctctaccat ttctgtgtgt tttccgataa cattattgct 721 acttctgttg tggttaattc tactgctctc aattccaagg cccctgagaa agttgtgttt 20 781 catcttgtga ctaatgagat caactatgct gcaatgaagg cttggttcgc cattaatatg 841 gacaacctca gaggagtcac tgtggaggtt cagaagttcg aggatttctc atggctgaat 901 gcttcctatg ttccggtcct caagcagctg caagactctg atacgcaaag ctattatttc 961 tetggacaca acgatgatgg gegeacteea ateaaattea ggaaceecaa gtatetttee 1021 atgctcaacc atcttaggtt ctacatccct gaagtgtttc ctgcgctgaa gaaggtggtc 25 1081 tttcttgatg atgatgttgt agttcagaag gatctttcat ctctcttttc gatcgattta 1141 aacaaaatg tgaacggggc tgttgagacc tgcatggaga ccttccaccg ctaccacaag 1201 tacttgaact attctcatcc tctcatacgc tcccactttg atccagatgc gtgtgggtgg 1261 gcgtttggaa tgaacgtctt tgatttagtt gagtggagga agagaaatgt gaccggcata 1321 taccactact ggcaagaaaa aaacgtggac cggaccttat ggaaactggg aacactacct 30 1381 ccaggacttc tgacatttta cgggttaaca gaggcactag aggcgtcctg gcatatcctg 1441 ggattgggat acacgaatgt ggatgctcgt gtgatagaga aaggagctgt tcttcacttc 1501 aatgggaact taaagccatg gttgaagatc gggatagaga agtacaaacc tttgtgggag 1561 agatacgttg attacacttc tccttttatg caacaatgca attttcattg a 35

Amino Acid Sequence of Sequence #6: (SEQ ID NO: 12) Genebank ID# NP\_565485 Positions 1-536 of NP\_565485.

1 mrrrggdsfr ragrrkisnv vwwvlsgial llfflilska ghieprpsip krryrndkfv
61 egmnmteeml sptsvarqvn dqialakafv viakesknlq fawdlsaqir nsqillssaa
121 trrspltvle sestirdmav llyqaqqlhy dsatmimrlk asiqaleeqm ssvsekssky
181 gqiaaeevpk slyclgvrlt tewfqnldlq rtlkersrvd skltdnslyh fcvfsdniia
241 tsvvvnstal nskapekvvf hlvtneinya amkawfainm dnlrgvtvev qkfedfswln
301 asyvpvlkql qdsdtqsyyf sghnddgrtp ikfrnpkyls mlnhlrfyip evfpalkkvv
361 fldddvvvqk dlsslfsidl nknvngavet cmetfhryhk ylnyshplir shfdpdacgw
421 afgmnvfdlv ewrkrnvtgi yhywqeknvd rtlwklgtlp pglltfyglt ealeaswhil
481 glgytnvdar viekgavlhf ngnlkpwlki giekykplwe ryvdytspfm qqcnfh

#### Sequence #7 (SEQ ID NO: 13)

Gene name: At2g30575

GeneBank accession # for reference: NM\_179819 GI:30684641

5 Nucleotide sequence of Sequence #7:

Positions 1-1833 of NM 179819.

1 atgaatcaag ttcgtcgttg gcagaggatt ctgatcctct cgctgctatt gttatctgtt 61 ttageteega ttgttttegt ttegaategg etcaagagea teaetteegt egatagagga 10 121 gaattcattg aagaattatc cgacattaca gataagaccg aggatgaact tagacttact 181 gctattgaac aggacgaaga aggcttgaag gagcctaaac gtattctgca ggatcgagat 241 tttaattctg tggttttgtc aaattcctct gataaaagta atgatactgt gcagtctaat 301 gagggagacc aaaaaaactt tctctcagaa gttgataagg gaaataatca caaaccaaag 361 gaggaacaag cagtttcaca gaaaaccaca gtaagctcga atgcggaggt gaaaatttca 15 421 gcaagagata ttcaacttaa tcataaaacg gaattccgac ccccttcaag taagagtgaa 481 aagaatacaa gggttcaact tgaaagagca acagatgaga gggtaaagga gatcagagac 541 aaaattatcc aagcgaaagc ctatctgaat ttggccctac ctgggaataa ctcccaaatc 601 gtaaaggagt tgagagttcg aacgaaagag ctggaacggg ctactggtga tactaccaag 661 gataaatatt tgccaaagag ctctcctaac agattgaagg ccatggaagt tgcgttatac 721 aaggtcagcc gtgcctttca caactgccct gccattgcta ccaaactcca agccatgact 20 781 tataaaaccg aagaacaagc tcgggcgcag aagaaacaag cagcatattt aatgcagctt 841 gcagcaagga ctaccccaaa agggcttcat tgtctctcaa tgcggttgac aacagaatat 901 tttaccetgg atcacgaaaa aaggcagett ttgcaacaaa gttataatga teetgatete 961 taccattacg tagtettete tgacaatgit ttggcetett eggttgttgt taactetaca 25 1021 atctcctcat caaaggaacc ggataaaata gtattccatg tggtgacaga ttcactcaat 1081 tacccagcaa tctcaatgtg gtttttacta aacccaagtg gcagagcttc aatccaaatc 1141 ctaaacattg atgaaatgaa tgtcctgcca ttgtaccatg ctgaattgct gatgaagcaa 1201 aattcaagtg acccaagaat catttcagcg ctcaaccatg cacgcttcta tctcccagat 1261 atcttcccag gtctaaacaa gatcgtactc ttcgatcatg atgtagtagt gcaaagggat 30 1321 ctaactagac tgtggagcct tgatatgacg gggaaagttg ttggagctgt agagacttgt 1381 cttgaaggtg atcettcata tegttegatg gacteattea ttaatttete agatgeatgg 1441 gtttctcaga aatttgatcc caaggcttgc acttgggcat tcgggatgaa tctatttgat 1501 ctcgaagaat ggagaagaca ggagttgact tctgtatacc tgaaatactt cgacctggga 1561 gtaaaaggac atctgtggaa agcaggggga ttgccagtag gttggttgac tttttcggg 35 1621 caaacgtttc cgttggaaaa gagatggaac gtgggtgggt taggtcacga atcaggactc 1681 agggcaageg acategaaca agcageggtt atacactaeg aegggateat gaaaccatgg 1741 ctggacatcg gtatagacaa gtacaagcgc tactggaaca tacatgtacc ttaccatcac 1801 cctcacttac aacggtgcaa cattcacgat tga

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Amino Acid Sequence of Sequence #7: (SEQ ID NO: 14) Genebank ID# NP\_850150 Positions 1-610 of NP\_850150.

1 mnqvrrwqri lilsllllsv lapivfvsnr lksitsvdrg efieelsdit dktedelrlt
61 aieqdeeglk epkrilqdrd fnsvvlsnss dksndtvqsn egdqknflse vdkgnnhkpk
121 eeqavsqktt vssnaevkis ardiqlnhkt efrppsskse kntrvqlera tdervkeird
181 kiiqakayln lalpgnnsqi vkelrvrtke leratgdttk dkylpksspn rlkamevaly
241 kvsrafhncp aiatklqamt ykteeqaraq kkqaaylmql aarttpkglh clsmrlttey
301 ftldhekrql lqqsyndpdl yhyvvfsdnv lassvvvnst issskepdki vfhvvtdsln

361 ypaismwfll npsgrasiqi Inidemnvlp lyhaellmkq nssdpriisa Inharfylpd 421 ifpglnkivl fdhdvvvqrd Itrlwsldmt gkvvgavetc legdpsyrsm dsfinfsdaw

481 vsqkfdpkac twafgmnlfd leewrrqelt svylkyfdlg vkghlwkagg lpvgwltffg

541 qtfplekrwn vgglghesgl rasdieqaav ihydgimkpw ldigidkykr ywnihvpyhh

601 phlqrcnihd

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### Sequence #8 (SEQ ID NO: 15)

Gene name: At2g46480

10 GeneBank accession # for reference: NM\_130212 GI:22326493

Nucleotide sequence of Sequence #8:

Positions 1-1587 of NM 130212.

1 atgactgatg cttgttgttt gaagggaaac gaggacaaaa tggttcctcg ttttggtcat 15 61 ggaacctgga taggaaaagc atttaatgat acaccagaga tgttgcatga aaggagtctg 121 agacaggaaa aaagattgga aagggctaat gagctgatga atgatgatag tctgcaaaag 181 cttgagacgg cagccatggc acgttccaga tctgtcgatt ctgcaccact aggaaactac 241 accattigga aaaatgaata ccggaggggc aagagtttig aagatatgtt acgtttgatg 301 caagatcaaa tcatcatggc acgagtttac agtggacttg caaagtttac aaacaatctc 361 gccttgcacc aagagataga aacacaacta atgaaactag cttgggagga agaatctact 20 421 gatattgatc aggagcagag agtacttgac agtataagag acatgggaca aatactggct 481 agagcacacg agcagctata tgaatgcaag ttggtgacaa ataagttgag agcaatgcta 541 caaacagttg aagatgaact cgaaaacgag cagacttata taacgttctt gactcagcta 601 gettecaagg cactaceaga tgetateeac tgettgacea tgegettgaa tetagagtat 661 catctcctgc ctttaccgat gagaaatttt ccaaggaggg agaatttgga gaatccaaaa 25 721 ctttaccact acgetetett etetgataat gtactggetg cateagttgt tgteaactee 781 acagteatga atgeacagga teetteaagg catgttttee acettgtgae tgataagete 841 aactttggag caatgagtat gtggtttctg ttgaaccctc ctggagaagc gaccatccat 901 gtccaaaggt ttgaagattt tacttggctc aactcatctt actctccagt tttgagtcag 30 961 ctcgagtcag cagctatgaa gaagttctac ttcaagacag cgaggtctga atcagttgaa 1021 teaggeteag aaaaceteaa gtaceggtae eegaaataca tgteaatget taaceacetg 1081 aggttetaea teectaggat etteecaaag ttggagaaaa tettgtttgt tgacgatgat 1141 gtggttgttc agaaggattt aactccccta tggtccattg atcttaaagg gaaagtgaat 1201 gaaaactttg atcccaagtt ctgcggatgg gcttatggga tgaacatctt cgacctgaaa 1261 gaatggaaga agaacaacat tacagaaact tatcactttt ggcaaaacct gaacgaaaac 35 1321 cggactetat ggaaactagg aacattgcca ccagggetea taacgtteta caatetgaca 1381 caaccacttc agagaaaatg gcacttactt ggactgggtt atgataaagg aatcgatgtc 1441 aagaagattg aaagatcagc tgttatacat tacaatggac acatgaaacc atggacagag 1501 atggggataa gcaagtatca gccatattgg acgaagtaca ccaattttga ccatccttac

Amino Acid Sequence of Sequence #8: (SEQ ID NO: 16) Genebank ID# NP\_182171 Positions 1-528 of NP 182171.

1561 atctttactt gcaggctgtt tgagtga

1 mtdaccikgn edkmvprfgh gtwigkafnd tpemihersi rgekrieran elmnddsigk

61 letaamarsr svdsaplgny tiwkneyrrg ksfedmlrim qdqiimarvy sglakftnnl

121 alhqeietql mklaweeest didqeqrvld sirdmgqila raheqlyeck lvtnklraml

181 gtvedelene gtyitfltgl askalpdaih cltmrlnley hllplpmrnf prrenlenpk

241 lyhyalfsdn vlaasvvvns tvmnaqdpsr hvfhlvtdkl nfgamsmwfl Inppgeatih

- 301 vqrfedftwl nssyspvlsq lesaamkkfy fktarsesve sgsenlkyry pkymsmlnhl
- 361 rfyiprifpk lekilfvddd vvvqkdltpl wsidlkgkvn enfdpkfcgw aygmnifdlk
- 421 ewkknnitet yhfwqnlnen rtlwklgtlp pglitfynlt qplqrkwhll glgydkgidv
- 481 kkiersavih ynghmkpwte mgiskyqpyw tkytnfdhpy iftcrlfe

# Sequence #9 (SEQ ID NO: 17)

Gene name: At3g01040

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10 GeneBank accession # for reference: NM\_110969 GI:30678269

Nucleotide sequence of Sequence #9: Positions 1-1602 of CDS of NM 110969.

15 1 atgcagette acatategee tagcatgaga agcattacga tategageag caatgagttt 61 attgatttga tgaagatcaa agtcgcagct cgtcacatct cttaccgaac tctcttccac 121 actatettaa teetegetti ettgttaeet tttgttttea teetaaeege tgttgttaee 181 cttgaaggtg tcaacaagtg ctcctctttt gattgtttcg ggaggcggct aggaccacgt 241 cttcttggta ggatagatga ttcagagcag agactagtta gagattttta caaaattcta 20 301 aatgaagtaa gcactcaaga aattccagat ggtttaaagc ttccagagtc ttttagtcaa 361 ctggtttcgg atatgaagaa caaccactat gatgctaaaa catttgccct cgtatttcga 421 gctatggtag agaagtttga aagggattta agggaatcca aatttgcaga actcatgaac 481 aagcactttg ctgcaagttc aattccaaaa ggaattcact gtctctcttt aagactaacc 541 gatgaatatt cetecaatge teatgeegg agacagette etteeegga getteteet 25 601 gttctctcag acaatgctta ccaccatttt gttctagcta cagataatat cttagctgca 661 toggttgtgg totcatotgc tgttcaatca tottcaaaac cogagaaaat tgtottccat 721 gttatcacag acaagaaaac ctatgcgggt atgcattctt ggtttgcact caattctgtt 781 gctcctgcga ttgttgaagt gaaaagcgtt catcagtttg attggttaac aagagagaat 841 gttccagttc ttgaagctgt ggaaagccat aacagtatca gaaattatta ccatgggaat 30 901 catattgctg gtgcaaacct cagcgaaaca acccctcgaa catttgcttc gaaactgcag 961 tcaagaagtc ccaaatacat atctttgctc aaccatctta gaatatatct accagagctt 1021 tttccgaact tagacaaggt agtgttctta gatgatgata tagtgataca gaaagattta 1081 tctccgcttt gggatattga ccttaacggg aaggttaatg gagctgtgga gacttgtcga 1141 ggagaagacg tatgggttat gtcaaagcgt cttaggaact acttcaattt ttctcacccq 1201 ctcatcgcaa agcatttaga tcccgaagaa tgtgcttggg cttatggaat gaatatcttt 35 1261 gatctacgga cttggaggaa gacaaatatc agagaaacgt atcattcttg gcttaaagag 1321 aatetgaagt egaatetaac aatgtggaaa ettggaacat tgeeteetge tetaatagea 1381 tttaaaggtc atgttcagcc aatagattcc tcttggcata tgcttggatt aggttatcag 1441 agcaagacca acttagaaaa tgcgaagaaa gctgcagtga ttcattacaa tggccaatca 40 1501 aagccgtggc ttgagatagg tttcgagcat ctcagaccat tctggacaaa atatgttaac

Amino Acid Sequence of Sequence #9: (SEQ ID NO: 18)
45 Genebank ID# NP\_186753
Positions 1-533 of NP\_186753.

1561 tactccaatg atttcattaa gaattgtcat atcttggaat ag

1 mqlhispsmr sitisssnef idlmkikvaa rhisyrtlfh tililafllp fvfiltavvt

- 61 legvnkcssf dcfgrrlgpr llgriddseq rlvrdfykil nevstqeipd glklpesfsq
- 121 lvsdmknnhy daktfalvfr amvekferdl reskfaelmn khfaassipk gihclslrlt
  - 181 deyssnahar rqlpspellp vlsdnayhhf vlatdnilaa svvvssavqs sskpekivfh

241 vitdkktvag mhswfalnsv apaivevksv hqfdwltren vpvleavesh nsirnyyhgn

301 hiaganiset tortfaskig srspkyisii nhiriyipel fpnidkvvfl dddivigkdi

361 splwdiding kvngavetcr gedvwvmskr irnyfnfshp liakhidpee cawaygmnif

421 dirtwrktni retyhswike niksnitmwk igtippalia fkghvqpids swhmigigyq

481 sktnlenakk aavihynggs kpwleigfeh Irpfwtkyvn ysndfiknch ile

### Sequence #10 (SEQ ID NO: 19)

Gene name: At3g02350

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GeneBank accession # for reference: NM 111102 GI:18396158 10

Nucleotide sequence of Sequence #10: Positions 1-1686 of CDS of NM 111102.

1 atggcggtgg ccttccgtgg aggccgggga ggcgtcggat ccggccaatc taccggactt

61 cgtagtttet tetectaceg gatetttate teegetttgt tetetttet etteetegee

121 actttctccg tcgttcttaa ctcctctcgt catcagcctc atcaggatca tacattgccg

181 agtatgggca acgcatatat gcagaggacg tttttggctt tgcaatcgga tccattgaaa

241 actaggttgg atctgataca caagcaagcc attgatcatt tgacactggt gaatgcgtat

301 gctgcttacg ctaggaaget aaagettgat gettetaage agettaaget ettegaagat

361 ttggctatca acttctcgga tttgcagtcg aaacctggtt tgaaatctgc tgtgtctgat

421 aatggtaatg ctcttgagga ggattcgttt aggcagcttg agaaagaagt gaaggataag

481 gtgaagacag cgaggatgat gatcgttgag tctaaagaga gttatgatac acagcttaaa

541 atccagaagt tgaaagatac aatctttgct gtccaagaac agttgacaaa ggctaagaaa

601 aacggtgcgg ttgctagctt gatttcagcc aagtcggttc ctaaaagtct tcattgtttg

661 gccatgaggc ttgtaggaga gaggatetet aatectgaga agtacaagga tgetecacet

721 gacccagccg cagaggatcc aactctttac cactatgcga ttttctctga taatgtcatt

781 gctgtgtctg ttgtggtgag atcggttgtg atgaacgctg aggagccatg gaagcatgtc

841 ttccatgtgg tgacagatcg gatgaatctc gcagccatga aggtgtggtt taagatgcgt

901 cctttggacc gtggtgccca tgttgagatt aaatccgtgg aggatttcaa gttcttaaac

961 tetteetatg egeeggtett gaggeagett gagtetgeea agttgeagaa gttttaettt

1021 gagaatcaag ctgagaacgc aactaaagat tcacataacc tcaagttcaa gaaccccaag

1081 tatctctcga tgttgaacca tctcagattt tacttaccag agatgtatcc gaagctgaat

1141 aagattttgt tettggacga tgatgttgtg gtgcagaaag acgtgactgg tttatggaaa

1201 atcaacttgg atggcaaggt gaatggagcc gttgagacat gttttggttc ttttcatcga

1261 tatggtcaat acttaaactt ctctcatcct ttgatcaaag agaactttaa ccccagtgcc

1321 tgtgcttggg cctttggaat gaacatattc gatctcaatg cctggagacg cgagaagtgc

1381 accgatcaat accattactg gcagaacctg aatgaagaca gaactctctg gaaattggga

1441 actotacete egggattgat cacattetat teaaagaega aateattgga eaaateatgg

1501 catgtacttg ggttaggcta taacccggga gtgagcatgg acgaaatcag aaatgcagga

1561 gtgattcatt acaatggaaa catgaaaccg tggctagaca ttgcgatgaa ccaatacaag

1621 totototoga ctaaatatgt tgataacgaa atggagtttg tgcagatgtg caattttggt 1681 ctctaa

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Amino Acid Sequence of Sequence #10: (SEQ ID NO: 20) Genebank ID# NP\_566170.1 Positions 1-561 of NP\_566170.

1 mavafrggrg gygsgqstgl rsffsyrifi salfsfifla tfsvvlnssr hqphqdhtlp
61 smgnaymqrt flalqsdplk trldlihkqa idhltlvnay aayarkikld askqlklfed
121 lainfsdlqs kpglksavsd ngnaleedsf rqlekevkdk vktarmmive skesydtqlk
181 iqklkdtifa vqeqltkakk ngavaslisa ksvpkslhcl amrlvgeris npekykdapp
241 dpaaedptly hyaifsdnvi avsvvvrsvv mnaeepwkhv fhvvtdrmnl aamkvwfkmr
301 pldrgahvei ksvedfkfln ssyapvlrql esaklqkfyf enqaenatkd shnlkfknpk
361 ylsmlnhlrf ylpemypkln kilfldddvv vqkdvtglwk inldgkvnga vetcfgsfhr
421 ygqylnfshp likenfnpsa cawafgmnif dlnawrrekc tdqyhywqnl nedrtlwklg
481 tlppglitfy sktksldksw hvlglgynpg vsmdeirnag vihyngnmkp wldiamnqyk
541 slwtkyvdne mefvqmcnfq I

15

# Sequence #11 (SEQ ID NO: 21)

Gene name: at3g25140

GeneBank accession # for reference: NM\_113418 GI:30687767

Nucleotide sequence of Sequence #11: Positions 1-1680 of CDS of NM 113418.

1 atggctaatc accaccgact tttacgcggc ggcggatctc cggccataat cggtggcaga 61 atcacactca cagetttege ttecactate geactettee tetteactet etecttette 25 121 ttcgcttcag attctaacga ttctcctgat ctccttcttc ccggtgttga gtactctaat 181 ggagtcggat ctagaagatc catgttggat atcaaatcgg atccgcttaa gccacggttg 241 attcagatcc ggaaacaagc tgatgatcat cggtcattag cattagctta tgcttcttac 301 gcgagaaagc ttaagctcga gaattcgaaa ctcgtcagga tcttcgctga tctttcgagg 361 aattacacgg atctgattaa caaaccgacg tatcgagctt tgtatgattc tgatggagcc 421 tcgattgaag aatctgtgct taggcaattt gagaaagaag ttaaggaacg gattaaaatg 30 481 actogicaag tgattgotga agotaaagag tottitigata atcagttgaa gattoagaag 541 ctgaaagata cgattttcgc tgttaacgaa cagttaacta atgctaagaa gcaaggtgcg 601 ttttcgagtt tgatcgctgc gaaatcgatt ccgaaaggat tgcattgtct tgctatgagg 661 ctgatggaag agaggattgc tcaccctgag aagtatactg atgaagggaa agatagaccg 35 721 cgggagctcg aggatccgaa tctttaccat tacgctatat tttcggataa tgtgattgcg 781 gcttcggtgg ttgtgaactc tgctgtgaag aatgctaagg agccgtggaa gcatgttttt 841 cacgttgtga ctgataagat gaatcttgga gctatgcagg ttatgtttaa actgaaggag 901 tataaaggag ctcatgtaga agttaaagct gttgaggatt atacgttttt gaactcttcg 961 tatgtgcctg tgttgaagca gttagaatct gcgaatcttc agaagtttta tttcgagaat 1021 aagctcgaga atgcgacgaa agataccacg aatatgaagt tcaggaaccc caagtattta 40 1081 tctatattga atcacttgag gttttattta cccgagatgt acccgaaact acataggata 1141 ctgtttttgg acgatgatgt ggttgtgcag aaggatttaa cgggtctgtg ggagattgat 1201 atggatggga aagtgaatgg agctgtagag acttgttttg ggtcgtttca tcggtacgct 1261 caatacatga atttctcaca tcctttgatc aaagagaagt ttaatcccaa agcatgtgcg 1321 tgggcgtatg gaatgaactt ctttgatctt gatgcttgga gaagagagaa gtgcacagaa 45 1381 gaatatcact actggcaaaa tctgaacgag aacagggctc tatggaaact ggggacgtta 1441 ccaccgggac tgatcacctt ttactcaacc acaaagccgc tggacaaatc atggcatgtg 1501 cttgggctgg gttacaatcc gagcattagc atggatgaga tccgcaacgc tgcagtggta 1561 cacttcaacg gtaacatgaa gccatggctt gacatagcta tgaaccagtt tcgaccactt 1621 tggaccaaac acgtcgacta tgacctcgag tttgttcagg cttgcaattt tggcctctga 50

Amino Acid Sequence of Sequence #11: (SEQ ID NO: 22) Genebank ID# NP\_189150 Positions 1-559 of NP\_189150.

1 manhhrlirg ggspaiiggr itltafasti alfiftlsff fasdsndspd llipgveysn
61 gvgsrrsmld iksdplkprl iqirkqaddh rslalayasy arklklensk lvrifadlsr
121 nytdlinkpt yralydsdga sieesvlrqf ekevkerikm trqviaeake sfdnqlkiqk
181 lkdtifavne qltnakkqga fssliaaksi pkglhclamr lmeeriahpe kytdegkdrp
241 reledpnlyh yaifsdnvia asvvvnsavk nakepwkhvf hvvtdkmnlg amqvmfklke
301 ykgahvevka vedytfinss yvpvlkqles anlqkfyfen klenatkdtt nmkfrnpkyl
361 silnhlrfyl pemypklhri lfldddvvvq kdltglweid mdgkvngave tcfgsfhrya
421 qymnfshpli kekfnpkaca waygmnffdl dawrrekcte eyhywqnlne nralwklgtl
481 ppglitfyst tkpldkswhv lglgynpsis mdeirnaavv hfngnmkpwl diamnqfrpl
541 wtkhvdydle fvqacnfgl

15

### Sequence #12 (SEQ ID NO: 23)

Gene name: At3g58790

GeneBank accession # for reference: NM 115741 GI:22331856

20 Nucleotide sequence of Sequence #12: Positions 1-1623 of CDS of NM 115741.

1 atgaagtttt acatatcagc gacggggatt aagaaggtta cgatatcaaa tcccggcgtc 61 ggaatcggta aaggaagcgg aggatgtgcg gctgcagcgg cggcgttagc agcgcggaga 121 ttetetagte geacgttgtt actgttgetg etgetgeteg etategteet ecettttate 25 181 ttcgtcaggt tcgcgtttct cgtcctcgaa tctgcctccg tttgcgattc accactcgat 241 tgcatgggac tcagactttt ccgtgggggc gacacatctc tgaaaattgg ggaagagttg 301 acacgggctc tagtggaaga gacgacagat catcaggacg ttaatggaag aggaacgaag 361 ggatcattgg agtcattcga cgaccttgtt aaggagatga cgttaaaacg ccgtgacata 421 agggcgtttg cttccgtgac taagaagatg ctgttgcaga tggaacgtaa agtccaatca 30 481 gcgaaacatc atgagttagt gtactggcat ttagcetete acggtattee taaaageete 541 cattgccttt ccctcagatt aactgaagag tactctgtaa atgcaatggc tcgaatgcgt 601 ttgcctccgc ctgagtccgt atcacgtctg accgacccat cttttcatca tattgtcctc 661 ctgactgaca atgtccttgc tgcctctgtc gtcatatcgt ctactgtaca aaacgctgtg 721 aatcccgaga agtttgtctt tcatattgtt accgataaga aaacctatac ccctatgcat 35 781 gettggtttg etateaacte tgetteatea eeagttgttg aagtaaaggg aetteateag 841 tatgattggc ctcaagaagt gaacttcaaa gttagagaga tgctggacat tcaccgctta 901 atttggagac gacattatca aaatttgaaa gactctgatt ttagttttgt tgagggtact 961 catgagcagt cettgeaage tetaaateet agetgeettg eeettttgaa ceatettege 1021 atttacattc ccaagctttt tccagatctc aacaagatag tgttgttgga tgatgatgta 40 1081 gtagtacaga gcgatctttc gtctttatgg gaaacggatc tcaacggtaa agttgttggt 1141 getgtegttg attegtggtg eggagacaac tgttgeeceg gaagaaaata caaagactat 1201 ttcaacttct cacatccttt gatctcatca aacttagttc aagaagactg tgcttggctt 1261 tetagtatga atgtetttga teteaaagee tggagacaaa eeaatattae tgaagettae 45 1321 totacatggc taagactcag tottaggtca ggactacaat tatggcaacc aggggcttta 1381 ccaccgacat tacttgcttt caaaggactt acacagtctc ttgaaccatc atggcacgtc 1441 getggaetag gttetegate egtaaaatee ceteaagaga ttetgaaate tgetteggtt 1501 ttacatttca gcggtccagc aaaaccgtgg ctagagatca gtaaccctga ggtacgatct 1561 ctttggtata gatacgtaaa ttcctccgac atcttcgtta gaaaatgcaa aatcatgaac 50 1621 tga

Amino Acid Sequence of Sequence #12: (SEQ ID NO: 24) Genebank ID# NP\_191438.2 Positions 1-540 of NP\_191438.

1 mkfyisatgi kkvtisnpgv gigkgsggca aaaaalaarr fssrtlllll Illaivlpfi
61 fvrfaflvle sasvcdspld cmglrlfrgg dtslkigeel tralveettd hqdvngrgtk
121 gslesfddlv kemtlkrrdi rafasvtkkm llqmerkvqs akhhelvywh lashgipksl
181 hclslrltee ysvnamarmr lpppesvsrl tdpsfhhivl ltdnvlaasv visstvqnav
241 npekfvfhiv tdkktytpmh awfainsass pvvevkglhq ydwpqevnfk vremldihrl
301 iwrrhyqnlk dsdfsfvegt heqslqalnp sclallnhlr iyipklfpdl nkivlldddv
361 vvqsdlsslw etdlngkvvg avvdswcgdn ccpgrkykdy fnfshpliss nlvqedcawl
421 sgmnvfdlka wrqtniteay stwlrlsvrs glqlwqpgal pptllafkgl tqslepswhv
481 aglgsrsvks pqeilksasv lhfsgpakpw leisnpevrs lwyryvnssd ifvrkckimn

# 15 <u>Sequence #13 (SEQ ID NO: 25)</u>

20

Gene name: At4g38270
GeneBank accession # for reference: NM\_119989 GI: 30691874
Nucleotide sequence of Sequence #13
Positions 1-2043 of CDS of NM 119989.

1 atgacgacgt tetetacatg egeogeettt ttategetgg tagtagtget acatgetgtt 61 catgteggtg gagecatttt agagteacaa geaceceaca gagaaettaa agettategt 121 ccgctgcaag ataataatct acaggaggtg tatgcttcct cagctgctgc agtgcactac 25 181 gatccagatc tgaaagatgt gaacatagtt gcgacataca gtgaccatta cggcaatata 241 cgccttggta gggtgaaaat gggggatctt tcaccttctt gggttttgga gaatcctgcc 301 tatcaagtta gccgcaaaac aaaaggttcg cagctagtta taccacggga ttcatttcaa 361 aatgatactg gaatggaaga taatgcaagc cattctacaa ctaatcagac tgatgaaagc 421 gaaaatcagt ttccaaacgt ggattttgca agcccagcaa aactgaagcg gcagatttta 30 481 cgtcaggaaa ggagaggtca acgaacttta gagctgatcc gacaagaaaa ggaaactgat 541 gagcagatgc aagaagcagc cattcagaag tcaatgagct ttgaaaaactc agtcataggg 601 aaatacagta tatggaggag agactatgag agcccaaatg ctgatgctat cttgaagctt 661 atgagagacc agatcataat ggcaaaagca tatgcaaata ttgccaaatc aaaaaatgta 721 accaatctgt acgttttctt gatgcagcag tgtggagaaa ataaacgtgt tataggtaaa 35 781 geaacetetg atgetgacet teetteaage getettgate aageaaaage catgggeeat 841 gcactctctc ttgcaaaaga cgagttatat gactgccatg aacttgcaaa aaagttccgg 901 gccatccttc agtccactga acgcaaagta gatggactga agaaaaaggg aaccttctta 961 attcagctag ctgccaaaac atttcccaag ccattgcatt gcctgagtct gcagctagcg 1021 gcagactatt ttattctagg tttcaatgaa gaggatgcag tgaaagagga tgtcagtcaa 40 1081 aagaagettg aagateette getetateae tatgegatet titeggataa egitetgget 1141 acatcagtgg tggtgaactc cactgtcttg aatgcaaagg aaccgcagag gcatgtgttc 1201 catatagtaa ctgacaaact gaattttggt gcaatgaaga tgtggtttcg catcaatgct 1261 cctgctgatg cgacgattca agttgaaaac ataaatgatt tcaagtggct gaactcctct 1321 tactgetetg ttetaeggea gettgaatet geaaggetga aagaataeta ttteaaagea 45 1381 aatcateett eateaatete agetggegea gataatetaa agtaeegeaa eecaaagtat 1441 ctatcgatgc tgaatcatct cagattctac cttcctgagg tttatccgaa gctggagaag 1501 attetette tagacgatga cattetegtg cagaaggace togcaccact atgggaaata 1561 gacatgcaag gaaaagtgaa tggtgcggtg gagacgtgca aggagagctt ccacagattt 1621 gacaagtace teaacttete aaateeaaag attteagaga attttgaege tggtgettgt 50 1681 gggtgggcat ttgggatgaa tatgtttgac ctgaaagagt ggaggaaacg gaacattaca

1741 gggatatatc actattggca agacttgaat gaagacagaa cactgtggaa gctgggatcg 1801 ttgccaccgg ggctgataac attttacaac ctgacgtatg caatggatag gagctggcac 1861 gtactagggc tgggatatga cccagcgcta aaccaaacag caatagagaa tgcagcggta 1921 gtgcattaca atgggaacta caagccatgg ctgggtttag cattcgccaa gtacaaaccg 1981 tactggtcca agtacgttga gtacgacaac ccttatctcc gacggtgcga catcaatgaa 2041 tga

Amino Acid Sequence of Sequence #13: (SEQ ID NO: 26)

Genebank ID# NP\_195540.2

Positions 1-680 of NP\_195540.

1 mttfstcaaf Islvvvlhav hvggailesq aphrelkayr plqdnnlqev yassaaavhy
61 dpdlkdvniv atysdhygni rlgrvkmgdl spswvlenpa yqvsrktkgs qlviprdsfq
121 ndtgmednas hsttnqtdes enqfpnvdfa spaklkrqil rqerrgqrtl elirqeketd
181 eqmqeaaiqk smsfensvig kysiwrrdye spnadailkl mrdqiimaka yaniaksknv
241 tnlyvflmqq cgenkrvigk atsdadlpss aldqakamgh alslakdely dchelakkfr
301 ailqsterkv dglkkkgtfl iqlaaktfpk plhclslqla adyfilgfne edavkedvsq
361 kkledpslyh yaifsdnvla tsvvvnstvl nakepqrhvf hivtdklnfg amkmwfrina
20 421 padatiqven indfkwlnss ycsvlrqles arlkeyyfka nhpssisaga dnlkyrnpky
481 lsmlnhlrfy lpevypklek ilfldddivv qkdlaplwei dmqgkvngav etckesfhrf
541 dkylnfsnpk isenfdagac gwafgmnmfd lkewrkrnit giyhywqdln edrtlwklgs
601 lppglitfyn ltyamdrswh vlglgydpal nqtaienaav vhyngnykpw lglafakykp
661 ywskyveydn pylrrcdine

Sequence #14 (SEQ ID NO: 27)

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Gene name: At5g15470
GeneBank accession # for reference: NM\_121551 GI:30685368
Nucleotide sequence of Sequence #14:
Positions 1-1599 of CDS of NM 121551.

1 atgcagette acatategee gagtatgaga agcattaega tttegageag caatgagttt 61 attgacttga tgaagatcaa ggtcgcagct cgtcacatct cttaccgaac tctcttccac 35 121 accatettaa teetegetti ettyttyeet titytiitea tieteaeege tytiyttaee 181 cttgagggtg tcaacaaatg ctcctccatt gattgtttag ggaggcggat aggtccacgt 241 cttcttggta gggtagatga ttcagagaga ctagctagag acttttataa aattctaaac 301 gaagtaagca ctcaagaaat tccagatggt ttgaagcttc caaattcttt tagtcaactt 361 gtttccgata tgaagaataa ccactatgat gcaaaaacat ttgctcttgt gctgcgagcc 421 atgatggaga agtttgaacg tgatatgagg gaatcgaaat ttgcagaact tatgaacaag 40 481 cactttgcag caagttccat tcccaaaggc attcattgtc tctctctaag actgacagat 541 gaatatteet ecaatgetea tgetegaaga eagetteett eaceagagtt tetecetgtt 601 ctttcagata atgettacca ccaetttatt ttgtccacgg acaatatttt ggctgcctca 661 gttgtggtct catccgctgt tcagtcatct tcaaaacccg agaaaattgt ctttcacatc 45 721 attacagaca agaaaaccta tgcgggtatg cattcatggt ttgcgcttaa ttctgttgca 781 ccagcaattg ttgaggttaa aggtgttcat cagtttgact ggttgacgag agagaatgtt 841 ccggttttgg aagctgtgga aagccataat ggtgtcaggg actattatca tgggaatcat 901 gtcgctgggg caaacctcac cgaaacaact cctcgaacat ttgcttcaaa attgcagtct 961 agaagtccaa aatacatatc tttgctcaac catcttagaa tatatatacc agagcttttc 1021 ccgaacttgg acaaggtggt tttcttagac gatgatatag ttgtccaggg agacttaact 50

1081 ccactttggg atgttgacct cggtggtaag gtcaatgggg cagtagagac ttgcaggggt 1141 gaagatgaat gggtgatgtc aaagcgttta aggaactact tcaatttctc tcacccgctc 1201 atcgcaaagc atttagatcc tgaagaatgt gcttgggcat atggtatgaa tatcttcgat 1261 ctacaagctt ggaggaaaac aaatatcaga gaaacgtatc actcttggct tagagagaat 1321 ctaaagtcaa atctgacaat gtggaaactt ggaaccttgc ctcctgctct tatcgcgttc 5 1381 aagggtcacg tacacataat agactcgtca tggcatatgc taggattagg ctaccagagc 1441 aagaccaaca tagaaaatgt gaagaaagca gcagtgatcc actacaatgg gcagtcaaag 1501 ccatggctgg agattggttt cgagcatctg cggccattct ggaccaaata cgtcaactac 1561 tcaaatgatt tcatcaagaa ctgtcacata ttggagtag 10 Amino Acid Sequence of Sequence #14: (SEQ ID NO: 28) Genebank ID# NP\_197051 Positions 1-532 of NP 197051. 15 1 mqlhispsmr sitisssnef idlmkikvaa rhisyrtlfh tililaflip fvfiltavvt 61 legvnkcssi delgrrigpr llgrvddser lardfykiln evstqeipdg lklpnsfsgl 121 vsdmknnhyd aktfalvlra mmekferdmr eskfaelmnk hfaassipkg ihcislrltd 181 eyssnaharr qlpspeflpv Isdnayhhfi Istdnilaas vvvssavqss skpekivfhi 241 itdkktyagm hswfalnsva paivevkgvh qfdwltrenv pvleaveshn gvrdyyhgnh 20 301 vaganltett prtfasklqs rspkyislln hiriyipelf pnldkvvfld ddivvggdlt 361 plwdvdlggk vngavetcrg edewvmskrl rnyfnfshpl iakhldpeec awaygmnifd 421 Iqawrktnir etyhswlren Iksnltmwkl gtlppaliaf kghvhiidss whmlglgygs 481 ktnienvkka avihyngqsk pwleigfehl rpfwtkyvny sndfiknchi le 25 Sequence #15 (SEQ ID NO: 29) Gene name: At5g54690 GeneBank accession # for reference: NM\_124850 GI:30696504 30 Nucleotide sequence of Sequence #15: Positions 1-1608 of CDS of NM\_124850. 1 atgcagttac atatatctcc gagcttgaga catgtgactg tggtcacagg gaaaggattg 61 agagagttca taaaagttaa ggttggttct agaagattct cttatcaaat ggtgttttac 121 tetetaetet tetteaettt tetteteega ttegtetttg tteteteeae egttgataet 35 181 atcgacggcg atccctctcc ttgctcctct cttgcttgct tggggaaaag actaaagcca 241 aagcttttag gaagaagggt tgattctggt aatgttccag aagctatgta ccaagtttta 301 gaacagcctt taagcgaaca agaactcaaa ggaagatcag atatacctca aacacttcaa 361 gatttcatgt ctgaagtcaa aagaagcaaa tcagacgcaa gagaatttgc tcaaaagcta 40 421 aaagaaatgg tgacattgat ggaacagaga acaagaacgg ctaagattca agagtattta 481 tatcgacatg tcgcatcaag cagcataccg aaacaacttc actgtttagc tcttaaacta 541 gccaacgaac actcgataaa cgcagcggcg cgtctccagc ttccagaagc tgagcttgtc 601 cctatgttgg tagacaacaa ctactttcac tttgtcttgg cttcagacaa tattcttgca 661 getteggttg tggetaagte gttggtteaa aatgetttaa gaceteataa gategttett 45 721 cacatcataa cggataggaa aacttatttc ccaatgcaag cttggttctc attgcatcct 781 ctgtctccag caataattga ggtcaaggct ttgcatcatt tcgattggtt atcgaaaggt 841 aaagtacccg ttttggaagc tatggagaaa gatcagagag tgaggtctca attcagaggt 901 ggatcatcgg ttattgtggc taataacaaa gagaacccgg ttgttgttgc tgctaagtta 961 caagetetea geectaaata caacteettg atgaateaca teegtattea tetaceagag

1021 ttgtttccaa gcttaaacaa ggttgtgttt ctagacgatg acattgtgat ccaaactgat

50

5	1081 ctttcacctc tttgggacat tgacatgaat ggaaaagtaa atggagcagt ggaaacatgt 1141 agaggagaag acaagtttgt gatgtcaaag aagttcaaga gttacctcaa cttctcgaat 1201 ccgacaattg ccaaaaactt caatccagag gaatgtgcat gggcttatgg aatgaatgtt 1261 ttcgacctag cggcttggag gaggactaac ataagctcca cttactatca ttggcttgac 1321 gagaacttaa aatcagacct gagtttgtgg cagctgggaa ctttgcctcc tgggctgatt 1381 gctttccacg gtcatgtcca aaccatagat ccgttctggc atatgcttgg tctcggatac 1441 caagagacca cgagctatgc cgatgctgaa agtgccgctg ttgttcattt caatggaaga 1501 gctaagcctt ggctggatat agcatttcct catctacgtc ctctctgggc taagtatctt 1561 gattcttctg acagatttat caagagctgt cacattagag catcatga
10	Amino Acid Sequence of Sequence #15: (SEQ ID NO: 30) Genebank ID# NP_200280
	Positions 1-535 of NM_200280.
15	1 mqlhispslr hvtvvtgkgl refikvkvgs rrfsyqmvfy slifftfilr fvfvlstvdt 61 idgdpspcss laclgkrlkp kllgrrvdsg nvpeamyqvl eqplseqelk grsdipqtlq 121 dfmsevkrsk sdarefaqkl kemvtlmeqr trtakiqeyl yrhvasssip kqlhclalkl 181 anehsinaaa rlqlpeaelv pmlvdnnyfh fvlasdnila asvvakslvq nalrphkivl 241 hiitdrktyf pmqawfslhp Ispaiievka lhhfdwlskg kvpvleamek dqrvrsqfrg
20	301 gssvivannk enpvvvaakl qalspkynsl mnhirihlpe lfpslnkvvf ldddiviqtd 361 lsplwdidmn gkvngavetc rgedkfvmsk kfksylnfsn ptiaknfnpe ecawaygmnv 421 fdlaawrrtn isstyyhwld enlksdlslw qlgtlppgli afhghvqtid pfwhmlglgy 481 qettsyadae saavvhfngr akpwldiafp hlrplwakyl dssdrfiksc hiras
25	The nucleotide and amino acid sequences of the ten <i>GALAT-LIKE</i> gene family members are shown as follows.
	Sequence #16 (SEQ ID NO:31)
30	Gene name: At1g02720 GeneBank accession # for reference: NM_100152, GI: 30678358 Nucleotide sequence of Sequence #16: Positions 1-1086 of CDS of NM_100152.
35	
<i></i>	1 atgcattgga ttacgagatt ctctgctttc ttctccgccg cattagccat gattctcctt     61 tctccttcgc tccaatcctt ttctccggcg gcagctatcc gatcatctca cccctacgcc     121 gacgaattca aaccccaaca aaactccgat tactcctcct tcagagaatc tccaatgttc     181 cgtaacgccg aacaatgcag atcttccggc gaagattccg gcgtctgtaa ccctaatctc
40	241 gtccacgtag ccatcactet cgacatcgat tacetecgtg getcaatege ageogteaat 301 tegateetee ageacteaat gtgcceteaa agegtettet tecaetteet egteteetee 361 gagteteaaa acetagaate tetgattegt tetaetttee ceaaattgae gaateteaaa 421 atttaetatt ttgcccetga gacegtaeag tetttgattt catetteegt gagaeaagee
45	481 ctagagcaac cgttgaatta cgccagaaat tacttggcgg atctgctcga gccttgcgtt 541 aagcgagtca tctacttgga ttcggatctc gtcgtcgtcg atgatatcgt caagctttgg 601 aaaacgggtt taggccagag aacaatcgga gctccggagt attgtcacgc gaatttcacg 661 aaatacttca ccggaggttt ttggtcagat aagaggttta acgggacgtt caaagggagg 721 aacccttgtt acttcaatac tggtgtaatg gtgattgatt tgaagaagtg gagacaattt

781 aggttcacga aacgaattga gaaatggatg gagattcaga agatagagag gatttatgag

- 841 cttggttctc ttcctccgtt tcttctggta tttgctggtc atgtagctcc gatttcacat
- 901 cggtggaatc aacatgggct tggtggtgat aatgttagag gtagttgccg tgatttgcat
- 961 totggtoctg tgagtttgct toactggtoa ggtagtggta agcoatggtt aagactcgat
- 1021 tocaagette catgleettt agacacattg tgggeacett atgatttgta taaacactee
  - 1081 cattga

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- Amino Acid Sequence of Sequence #16: (SEQ ID NO: 32)
  Genebank ID# NP\_171772
  Positions 1-361.
- 15 1 mhwitrfsaf fsaalamill spslqsfspa aairsshpya defkpqqnsd yssfrespmf
  - 61 rnaeqcrssg edsgvcnpnl vhvaitldid ylrgsiaavn silqhsmcpg svffhflvss
  - 121 esqnleslir stfpkltnlk iyyfapetvq slisssvrqa leqplnyarn yladllepcv
  - 181 krviyldsdl vvvddivklw ktglgqrtig apeychanft kyftggfwsd krfngtfkgr
  - 241 npcyfntgvm vidlkkwrqf rftkriekwm eigkieriye Igslppfllv faghvapish
- 20 301 rwnqhglggd nvrgscrdih sgpvslihws gsgkpwirld skipcpidti wapydlykhs 361 h

#### Sequence #17 (SEQ ID NO:33)

Gene name: At1g13250

- 25 GeneBank accession # for reference: NM\_101196, GI:30683194
  - Nucleotide sequence of Sequence #17: Positions 1-1038 of CDS of NM 101196.
- 30 1 atgtcttctc tgcgtttgcg tttatgtctt cttctactct tacctatcac aattagctgc
  - 61 gtcacagtca ctctcactga cctccccgcg tttcgtgaag ctccggcgtt tcgaaacggc
  - 121 agagaatget ccaaaacgae atggatacet teggateaeg aacaeaacee ateaateate
  - 181 cacategeta tgactetega egeaatttae eteegtgget eagtegeegg egtettetee
  - 241 gttctccaac acgettettg teetgaaaac ategttttee actteatege cacteacegt
  - 301 cgcagcgccg atctccgccg cataatctcc tcaacattcc catacctaac ctaccacatt
    - 361 taccattttg accetaacet cgtccgcage aaaatatett cetetatteg tegtgettta
    - 421 gaccaaccgt taaactacgc teggatetac etegeegate teeteecaat egeegteege
    - 481 cgcgtaatct acttcgactc cgatctcgta gtcgtcgatg acgtggctaa actctggaga
    - 541 atcgatctac gtcggcacgt cgtcggagct ccggagtact gtcacgcgaa tttcactaac
    - 601 tacttcactt caagattctg gtcgagtcaa ggttacaaat cggcgttgaa agataggaaa
      - 661 ccgtgttatt tcaacaccgg agtgatggtg attgatctcg gaaaatggag agaaaggaga
      - 721 gtcacggtga agctagagac atggatgagg attcaaaaac gacatcgtat ttacgaattg
      - 781 ggatctttgc ctccgtttct gctcgttttc gccggagatg ttgagccggt ggagcatagg
    - 841 tggaatcagc atggtcttgg tggtgataac ttggaaggac tttgccggaa tttgcatcca
    - 901 ggtccggtga gtttgttgca ttggagcggg aaagggaaac catggctaag gcttgactcg
    - 961 agacgaccgt gtccgttgga ttcgttatgg gctccttatg atttgtttcg ttattcaccg
    - 1021 ttgatctctg atagctga

Amino Acid Sequence of Sequence #17: (SEQ ID NO: 34) Genebank ID# NP\_563925 Positions 1-345.

- 1 msslrlrlcl illipitisc vtvtltdlpa freapafrng recskttwip sdhehnpsii 61 hiamtldaiy irgsvagyfs vlqhascpen ivfhfiathr rsadirriis stfpyltyhi
  - 121 yhfdpnlvrs kisssirral dqplnyariy ladllpiavr rviyfdsdlv vvddvaklwr
  - 181 idlrrhvvga peychanftn yftsrfwssq gyksalkdrk pcyfntgvmv idlgkwrerr
  - 241 vtvkletwmr iqkrhriyel gslppfllvf agdvepvehr wnqhglggdn leglcrnlhp
  - 301 gpvslihwsg kgkpwlrids rrpcpldslw apydlfrysp lisds

### Sequence #18 (SEQ ID NO:35)

Gene name: At1g19300

GeneBank accession # for reference: NM\_101787, GI:30686302

Nucleotide sequence of Sequence #18: Positions 1-1056 of CDS of NM\_101787.

1 atgteceaac atettettet teteattete etetegetae ttettettea taaacceatt

- 61 tecgecacta caattattea aaaatteaaa gaageeeeac agttttacaa ttetgeagat
- 20 121 tgccccttaa tcgatgactc cgagtccgac gatgacgtgg tcgccaaacc aatcttctgc
  - 181 teacgtegag etgtecaegt ggegatgaea etegaegeeg cetacatteg tggeteagte
  - 241 geogetette teteogreet ceaacactet tetteteete aaaacattet ttteeactte
  - 301 gtcgcctctg cttccgccga cgcttcttcc ttacgagcca ccatatcctc ctctttccct
  - 361 tacettgatt teacegteta egtetteaae gteteeteeg tetetegeet tateteetee
- 25 421 totatccgct ccgcactaga ctgtccttta aactacgcaa gaagctacct cgccgatctc
  - 481 etecetecet gegteegeeg egtegtetae etagaeteeg atetgateet egtegaegae
  - 541 atagcaaaac tcgccgccac agatctcggc cgtgattcag tcctcgccgc gccggaatac
  - 601 tgcaacgcca atttcacttc atacttcaca tcaaccttct ggtctaatcc gactctctct
  - 661 ttaaccttcg ccgatcggaa agcatgctac ttcaacactg gagtcatggt gatcgatctt
- 721 teceggtgge gegaaggege gtacaegtea egeategaag agtggatgge gatgeaaaag
- 781 agaatgagaa tttacgagct tggttcgtta ccaccgtttt tattggtttt tgccggtttg
  - 841 attaaaccgg ttaatcatcg gtggaaccaa cacggtttag gaggtgataa tttcagagga
  - 901 ctgtgtagag atctccatcc tggtccggtg agtctgttgc attggagtgg gaaaggtaag
  - 961 ccatgggcta ggcttgatgc tggtcggcct tgtcctttag acgcgctttg ggctccgtat
- 35 1021 gatcttcttc aaacgccgtt cgcgttggat tcttga

Amino Acid Sequence of Sequence #18: (SEQ ID NO: 36) Genebank ID# NP\_564077 Positions 1-351.

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- 1 msqhllllil Isllllhkpi sattiiqkfk eapqfynsad cpliddsesd ddvvakpifc
- 61 srravhvamt Idaayirgsv aavlsvlqhs scpenivfhf vasasadass Iratisssfp
- 121 vldftyyyfn yssysrliss sirsaldcpl nyarsyladl lppcyrryyy ldsdlilydd
- 181 iaklaatdlg rdsvlaapey cnanftsyft stfwsnptls ltfadrkacy fntgvmvidl
- 241 srwregayts rieewmamgk rmriyelgsl ppfllvfagl ikpvnhrwng hglggdnfrg
  - 301 lcrdlhpgpv slihwsgkgk pwarldagrp cpldalwapy dligtpfald s

### Sequence #19 (SEQ ID NO:37)

Gene name: At1g24170

GeneBank accession # for reference: NM\_102263, GI:30688765

Nucleotide sequence of Sequence #19: Positions 1-1182 of CDS of NM\_102263.

1 atgtcgtcgc gtttttcttt gacggtggtg tgtttgattg ctctgttacc gtttgttgtt 61 ggtatacggt tgattccggc gaggatcacg agtgtcggtg atggcggcgg cggaggaggt 10 121 aataatgggt ttagtaaact tggtccgttt atggaagctc cggagtatag aaacggcaag 181 gagtototat cttcatcagt gaacagagag aacttcgtgt cgtcttcttc tagttctaat 241 gatecttege ttgttcacat egetatgact ttggactcag agtateteeg tggatcaate 301 geageegtte attetgttet tegeeaegeg tettgteeag agaaegtett etteeattte 361 atcgctgctg agtttgactc tgcgagtcct cgtgttctga gtcaactcgt gaggtcgact 15 421 tttccttcgt tgaactttaa agtctacatt tttagggaag atacggtgat caatctcata 481 tettettega ttagactage tttggagaat eegttgaaet atgeteggaa etatetegga 541 gatattettg ategaagtgt tgaacgagte atttatettg acteggatgt tataactgtg 601 gatgatatca caaagetttg gaacaeggtt ttgacegggt caegagtcat eggageteeg 661 gagtattgtc acgcgaactt cactcagtat ttcacttccg ggttctggtc agacccggct 721 ttaccgggtc taatctcggg tcaaaagcct tgctatttca acacaggagt gatggtgatg 20 781 gatcttgtta gatggagaga agggaattac agagagaagt tagagcaatg gatgcaattg 841 cagaagaaga tgagaatcta cgatcttgga tcattaccac cgtttctttt ggtgtttgcg 901 ggtaatgttg aagctattga tcatagatgg aaccaacatg gtttaggagg agacaatata 961 cgaggaagtt gtcggtcatt gcatcctggt cctgtgagct tgttgcattg gagtggtaaa 1021 ggtaagceat gggttagact tgatgagaag aggcettgte egttggatea tetttgggag 25 1081 ccatatgatt tgtataagca taagattgag agagctaaag atcagtctct gcttgggttt 1141 gcttctctgt cggagttgac tgatgattca agcttcttgt ga

- Amino Acid Sequence of Sequence #19: (SEQ ID NO: 38) Genebank ID# NP\_173827 Positions 1-393.
- 1 mssrfsltvv cliallpfvv girliparit svgdgggggg nngfsklgpf meapeyrngk
  61 ecvsssvnre nfvssssssn dpslvhiamt ldseylrgsi aavhsvlrha scpenvffhf
  121 iaaefdsasp rvlsqlvrst fpslnfkvyi fredtvinli sssirlalen plnyarnylg
  181 dildrsverv iyldsdvitv dditklwntv ltgsrvigap eychanftqy ftsgfwsdpa
  241 lpglisgqkp cyfntgvmvm dlvrwregny rekleqwmql qkkmriydlg slppfllvfa
  301 gnveaidhrw nqhglggdni rgscrslhpg pvsllhwsgk gkpwvrldek rpcpldhlwe
  361 pydlykhkie rakdqsllgf aslseltdds sfl

## Sequence #20 (SEQ ID NO:39)

Gene name: At1g70090

GeneBank accession # for reference: NM\_105677, GI:30697975

Nucleotide sequence of Sequence #20:
 Positions 1-1173 of CDS of NM 105677.

1 atgcggttgc gttttccgat gaaatctgcc gttttagcgt ttgctatctt tctggtgttt 61 attecactgt tttccgtcgg tatacggatg attccgggaa gactcaccgc cgtatccgcc 10 121 accgtcggaa atggctttga tctggggtcg ttcgtggaag ctccggagta cagaaacggc 181 aaggagtgcg tgtctcaatc gttgaacaga gaaaacttcg tgtcgtcttg cgacgcttcg 241 ttagttcatg tagctatgac gcttgactcg gagtacttac gtggctcaat cgcagccgta 301 cattcaatgc teegecaege gtegtgteea gaaaaegtet tetteeatet eategetgea 361 gagtttgacc cggcgagtcc acgcgttctg agtcaactcg tccgatctac tttcccgtcg 15 421 ctaaacttca aagtctacat tttccgggaa gatacggtga tcaaccttat ctcttcttca 481 atcagacaag ctttagagaa tccattgaac tatgctcgga actacctcgg agatattctt 541 gatccatgcg tagacagagt catttaccta gactcggaca tcatcgtcgt cgatgacata 601 acaaagcttt ggaacacgag tttgacaggg tcaagaatca tcggagctcc ggagtattgt 661 cacgctaact tcacaaagta cttcacttca ggtttctggt ccgacceggc tttacceggt 20 721 ttcttctcgg gtcgaaagcc ttgttatttc aacacgggtg tgatggtgat ggatctagtt 781 agatggagag aaggaaacta cagagaaaag cttgaaactt ggatgcagat acagaagaag 841 aagagaatet acgatttggg ttetttgeet eegtttette ttgtettege agggaacgtt 901 gaagcaattg atcataggtg gaaccaacat ggtttaggag gagacaatgt acgaggaagt 961 tgtaggtctt tgcataaagg accagtgagt ttgttgcatt ggagtggtaa aggtaagcca 1021 tgggtgagac ttgatgagaa gagaccgtgt ccgttggatc atttatggga accgtatgat 25 1081 ttatatgagc ataagattga aagagctaaa gatcagtctt tgttcgggtt ctcttctttg 1141 totgagttaa cagaagatto aagottttto tga

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Amino Acid Sequence of Sequence #20: (SEQ ID NO: 40) Genebank ID# NP\_564983 Positions 1-390.

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1 mrlrfpmksa vlafaiflvf iplfsvgirm ipgrltavsa tvgngfdlgs fveapeyrng 61 kecvsqslnr enfvsscdas lvhvamtlds eylrgsiaav hsmlrhascp envffhliaa 121 efdpasprvl sqlvrstfps lnfkvyifre dtvinlisss irqalenpln yarnylgdil

181 dpcvdrviyl dsdiivvddi tklwntsltg sriigapeyc hanftkyfts gfwsdpalpg

241 ffsgrkpcyf ntgvmvmdlv rwregnyrek letwmqiqkk kriydlgslp pfllvfagnv
301 eaidhwngh gladdryrgs crelbkapys llbwsgkgkp ywrldolyng pldblygg

301 eaidhrwngh glggdnvrgs crslhkgpvs llhwsgkgkp wyrldekrpc pldhlwepyd

361 lyehkierak dqslfgfssl seltedssff

#### Sequence #21 (SEQ ID NO:41)

Gene name: At3g06260

GeneBank accession # for reference: NM\_111501, GI:18397517

- Nucleotide sequence of Sequence #21: Positions 1-1056 of CDS of NM\_111501.
  - 1 atggceteaa ggageetete etataeaeaa eteetaggee teetgteett tataeteete
  - 61 ttggtcacaa ccaccactat ggcggttcgt gttggagtca ttcttcataa gccttctgct
- 10 121 ccaactette etgtttteag agaageeeg gettttegaa aeggtgatea atgegggaet
  - 181 cgtgaggetg atcagattca tatcgccatg actctcgaca caaactacct ccgtggcaca
  - 241 atggctgccg ttttgtctct ccttcaacat tccacttgcc ctgaaaacct ctcttttcat
  - 301 tteetgteee tteeteattt egaaaaegae etttteacea geateaaate aacettteet
  - 361 tacctaaact tcaagattta tcagtttgat ccaaacctcg tccgcagcaa gatatcgaaa
  - 421 tecateagge aageeettga teageetett aactaegeaa gaatetaeet egeggatate
    - 481 atccctagca gcgttgacag gatcatctac ttagactcag acctcgttgt ggtagacgac
    - 541 atagagaage tgtggcatgt ggagatggaa ggtaaagtgg tggctgctcc cgagtactgc
    - 601 cacgcaaact tcacccatta tttcacaaga actttctggt cagacccggt attggtcaaa
  - 661 gttcttgaag gaaaacgtcc gtgttatttc aacacagggg tgatggttgt ggatgtaaac 721 aaatggagga aaggaatgta tacacagaag gtagaagagt ggatgacaat tcagaagcag
  - 721 aaalggagga aaggaalgta tacacagaag glagaagagt ggalgacaat icagaagca 781 aagaggatat accatttggg atcattacct ccgtttctgc tgatattcgc cggtgatata
    - 841 aaagcggtta atcataggtg gaaccagcat ggtctaggag gtgataattt cgaaggaaga
    - 901 totagaacgt tocatccggg accgataagt cttcttcact ggagtggaaa agggaagcca
    - 961 tggttaagac tagattcaag gaagcettgt atcgttgate atctatggge accgtatgat
- 25 1021 ctgtaccgtt catcaagaca ttcattagaa gagtag
  - Amino Acid Sequence of Sequence #21: (SEQ ID NO: 42)
- 30 Genebank ID# NP\_187277
  - Positions 1-351.

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- 1 masrslsytg ligilsfill lyttttmavr vgvilhkpsa ptlpyfreap afrngdqcgt
- 61 readgihiam tldtnylrgt maavlsligh stepenisfh fisiphfend lftsikstfp
- 121 ylnfkiyqfd pnlvrskisk sirqaldqpl nyariyladi ipssvdriiy ldsdlvvvdd
- 181 ieklwhveme gkvvaapeyc hanfthyftr tfwsdpvlvk vlegkrpcyf ntgvmvvdvn
- 241 kwrkgmytak veewmtiaka kriyhlaslp pfllifagdi kavnhrwnah glagdnfegr
- 301 crtlhpgpis Ilhwsgkgkp wirldsrkpc ivdhlwapyd lyrssrhsle e

## Sequence #22 (SEQ ID NO:43)

Gene name: At3g28340

GeneBank accession # for reference: NM\_113753, GI:30689155

Nucleotide sequence of Sequence #22: Positions 1-1098 of CDS of NM\_113753.

1 atgatgtctg gttcaagatt agcctctaga ctaataataa tcttctcaat aatctccaca 61 tetttettea eegttgaate gattegaeta tteeetgatt eattegaega tgeatettea 121 gatttaatgg aagctccagc atatcaaaac ggtcttgatt gctctgtttt agccaaaaac 10 181 agactettgt tagettgtga tecateaget gtteatatag etatgaetet agateeaget 241 tacttgcgtg gcacggtatc tgcagtacat tccatcctca aacacacttc ttgccctgaa 301 aacatettet teeaetteat tgettegggt acaagteagg gtteeetege caagaceeta 361 teetetettt tteettettt gagttteaaa gtetataeet ttgaagaaac eaeggteaag 421 aatctaatct cttcttctat aagacaagct cttgatagtc ctttgaatta cgcaagaagc 15 481 tacttateeg agattettte ttegtgtgtt agtegagtga tttatetega tteggatgtg 541 attgtggtcg atgatattca gaaactatgg aagatttctt tatccgggtc aagaacaatc 601 gatacaccag agtattacca cacaaatttc accaaatact tcacagatag tttctggtcc 661 gatcaaaaac tctcgagtgt cttcgattcc aagactcctt gttatttcaa cacaggagtg 721 atggttatcg atttagagcg atggagagaa ggagattaca cgagaaagat cgaaaactgg 20 781 atgaagattc agaaagaaga taagagaatc tacgaattgg gttctttacc accgtttctt 841 ctagtgtttg gtggtgatat tgaagctatt gatcatcaat ggaaccaaca cggtctcggt 901 ggagacaaca ttgtgagtag ttgtagatct ttgcatcctg gtccggttag tttgatacat 961 tggagtggta aagggaagcc atgggttagg cttgatgatg gtaagccttg tccaattgat 1021 tatctttggg ctccttatga tcttcacaag tcacagaggc agtatcttca atacaatcaa 25

- Amino Acid Sequence of Sequence #22: (SEQ ID NO: 44)
  Genebank ID# NP\_189474
  Positions 1-365.
- 1 mmsgsrlasr liiifsiist sfftvesirl fpdsfddass dlmeapayqn gldcsvlakn
  61 rlllacdpsa vhiamtldpa ylrgtvsavh silkhtscpe niffhfiasg tsqgslaktl
  121 ssvfpslsfk vytfeettvk nlisssirqa ldsplnyars ylseilsscv srviyldsdv
  181 ivvddiqklw kislsgsrti gapeychanf tkyftdsfws dqklssvfds ktpcyfntgv
  241 mvidlerwre gdytrkienw mkiqkedkri yelgslppfl lvfggdieai dhqwnqhglg
  301 gdnivsscrs lhpgpvslih wsgkgkpwvr lddgkpcpid ylwapydlhk sqrqylqynq
  361 eleil

#### Sequence #23 (SEQ ID NO:45)

1081 gagttagaaa ttctttga

- Gene name: At3g50760
  GeneBank accession # for reference: NM\_114936, GI:18409176
  Nucleotide sequence of Sequence #23:
  Positions 1-1026 of CDS of NM\_114936.
- 1 atgcactcga agtttatatt atatctcagc atcctcgccg tattcaccgt ctctttcgcc

5	61 ggcggcgaga gattcaaaga agctccaaag ttcttcaact ccccggagtg tctaaccatc 121 gaaaacgatg aagatttcgt ttgttcagac aaagccatcc acgtggcaat gaccttagac 181 acagcttacc tccgtggctc aatggccgtg attctctccg tcctccaaca ctcttcttgt 241 cctcaaaaca ttgttttcca cttcgtcact tcaaaacaaa gccaccgact ccaaaactac 301 gtcgttgctt cttttcccta cttgaaattc cgaatttacc cttacgacgt agccgccatc 361 tccggcctca tctcaacctc catccgctcc gcgctagact ctccgctaaa ctacgcaaga 421 aactacctcg ccgacattct tcccacgtgc ctctcacgtg tcgtatacct agactcagat 481 ctcatactcg tcgatgacat ctccaagctc ttctccactc acatccctac cgacgtcgtt
10	541 ttagccgcgc ctgagtactg caacgcaaac ttcacgactt actttactcc gacgttttgg 601 tcaaaccett ctctctccat cacactatcc ctcaaccgcc gtgctacacc gtgttacttc 661 aacaccggag tgatggtcat cgagttaaag aaatggcgag aaggagatta cacgaggaag 721 atcatagagt ggatggagtt acaaaaacgg ataagaatct acgagttagg ctctttacca 781 ccgttttac ttgtcttcgc cggaaacata gctccggtag atcaccggtg gaaccaacac
15	841 ggtttaggag gagataattt tagaggactg tgtcgagatt tgcatccagg tccagtgagt 901 ttgttgcatt ggagtgggaa agggaagcca tgggtaaggt tagatgatgg tcgaccttgc 961 ccgcttgatg cactttgggt tccatatgat ttgttagagt cacggttcga ccttatcgag 1021 agttaa
20	Amino Acid Sequence of Sequence #23: (SEQ ID NO: 46) Genebank ID# NP_190645 Positions 1-341.
25	1 mhskfilyls ilavftvsfa ggerfkeapk ffnspeclti endedfvcsd kaihvamtld 61 taylrgsmav ilsvlqhssc pqnivfhfvt skqshrlqny vvasfpylkf riypydvaai 121 sglistsirs aldsplnyar nyladilptc Isrvvyldsd lilvddiskl fsthiptdvv 181 laapeycnan fttyftptfw snpslsitls Inrratpcyf ntgvmvielk kwregdytrk 241 iiewmelqkr iriyelgslp pfllvfagni apvdhrwnqh glggdnfrgl crdlhpgpvs
30	301 Ilhwsgkgkp wvrlddgrpc pldalwvpyd llesrfdlie s  Sequence #24 (SEQ ID NO:47)
35	Gene name: At3g62660 GeneBank accession # for reference: NM_116131, GI:30695642 Nucleotide sequence of Sequence #24: Positions 1-1086 of CDS of NM_116131.
40	1 atgetttgga teatgagatt eteeggttta tteteegeeg etttggttat eategteete 61 teteettete teeaategtt teeteeaget gaagetatea gateetetea tetegaeget 121 taeeteegtt teeeteete egateeaeeg eegeatagat teteetteag aaaageteet 181 gtttteegea atgeegeega ttgegeegee geagatateg atteeggegt etgtaaeeet

181 gitticegea atgeegeega ttgegeegee geagatateg atteeggegt etgtaaecet
241 teettggtee aegtegegat tactetegat ttegagtace tgegtggete aategeegee
301 giteattega tteteaagea etegtegtgt eeegagageg tettetteea titeetegte
361 teegagaetg aeetagaate ettgattegt tegaetitte eegaattgaa attaaaggit
421 tactaetteg ateeggagat tgtaeggaeg etgateteaa eeteegtgag aeaagegete
481 gageageegt tgaattaege tagaaattae etagetgaee ttetegagee ttgegtgegt
541 egegtgatet aeetagatte egatetaate gtegtegaeg aeategeaaa getetggatg
601 aegaaaetgg gategaaaae gateggaget eeegagtaet gteaegegaa etteaeaaag
50 661 tatticaeae eggegttetg gteegaegag aggitteteeg gagettitete eggaggaaa

- 721 ccgtgctact tcaacacggg agtgatggtg atggatctag agagatggag gcgcgtaggg 781 tacacggagg tgatagagaa atggatggag attcagaaga gtgataggat ttacgagctg 841 ggatcattgc cgccgttctt gttggtgttc gccggagaag tagctccgat agagcatcgg 901 tggaaccagc atgggcttgg tggagataac gtgagaggaa gctgtagaga tttacatccc 961 ggtccggtta gcttgcttca ttggtccggt agtggtaaac cgtggtttcg gttagattcg 1021 agacggcctt gtccacttga tactctttgg gcaccttatg atttgtatgg acactactct 1081 cgctga
- Amino Acid Sequence of Sequence #24: (SEQ ID NO: 48)
  Genebank ID# NP\_191825
  Positions 1-361.
- 1 mlwimrfsgl fsaalviivl spslqsfppa eairsshlda ylrfpssdpp phrfsfrkap
  61 vfrnaadcaa adidsgvcnp slvhvaitld feylrgsiaa vhsilkhssc pesvffhflv
  121 setdleslir stfpelklkv yyfdpeivrt listsvrqal eqplnyarny ladllepcvr
  181 rviyldsdli vvddiaklwm tklgsktiga peychanftk yftpafwsde rfsgafsgrk
  241 pcyfntgvmv mdlerwrrvg yteviekwme iqksdriyel gslppfllvf agevapiehr
  301 wnqhglggdn vrgscrdlhp gpvsllhwsg sgkpwfrlds rrpcpldtlw apydlyghys
  361 r

#### Sequence #25 (SEQ ID NO:49)

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Gene name: At4g02130
25 GeneBank accession # for reference: NM\_116445, GI:18411845
Nucleotide sequence of Sequence #25:
Positions 1-1041 of CDS of NM\_116445.

1 atgetttgga taacgagatt tgetggatta tteteegeeg egatggeagt gategtgtta 61 teteogtege tteagteatt teeteeggeg geggeaatee gttettetee ateacegate 30 121 ttcagaaaag ctccagcggt gttcaacaac ggcgacgaat gtctctcctc cggcggcgtc 181 tocaatecut cuttouteca cutgocuate acuttagaeg tagagtaeet gegtggetea 241 atcgcagccg ttaactcgat cettcagcac teggtgtgtc cagagagcgt ettettccac 301 ttcatcgccg tctccgagga aacaaacctg ttggagtcgc tggtgagatc ggttttcccg 361 agactgaaat tcaatattta cgattttgcc cctgagacag ttcgtggttt gatttcttct 35 421 tccgtgagac aagetetega geagectetg aactaegeta gaagetaett ageggatetg 481 ctggagcett gtgttaaccg tgtcatatac ttggattcgg atcttgtcgt cgtcgatgac 541 atcgctaagc tttggaaaac tagcctaggc tcgaggataa tcggagctcc ggagtattgt 601 cacgcgaatt tcacgaaata cttcaccgga ggattctggt cggaggagag attctccggt 661 acctttagag ggaggaagcc atgttacttc aacacaggtg tgatggtgat agatcttaag 40 721 aaatggagaa gaggtggtta cacgaaacgt atcgagaaat ggatggagat tcagagaaga 781 gagaggattt acgaactagg ctcgcttcca ccgtttcttc tagttttctc cggtcacgtg 841 geteceatet eteaceggtg gaaceageat ggaettggtg gtgacaatgt tagaggtage 901 tgtcgtgatt tgcatcctgg tcctgtgagt ttgctgcatt ggtctggtag tggcaagccc 961 tggataagac tcgattccaa acggccttgt cccttagacg cattatggac gccttacgac 45 1021 ttgtatcgac attcgcattg a

Amino Acid Sequence of Sequence #25: (SEQ ID NO: 50) Genebank ID# NP\_192122 Positions 1-346.

- 5 1 mlwitrfagl fsaamavivl spslqsfppa aairsspspi frkapavfnn gdeclssggv
  - 61 cnpslvhvai tldveylrgs iaavnsilqh svcpesvffh fiavseetnl leslvrsvfp
  - 121 rlkfniydfa petvrgliss svrqaleqpl nyarsyladl lepcvnrviy ldsdlvvvdd
  - 181 iaklwktslg sriigapeyc hanftkyftg gfwseerfsg tfrgrkpcyf ntgvmvidlk
  - 241 kwrrggytkr iekwmeiqrr eriyelgslp pfllvfsghv apishrwnqh glggdnvrgs
  - 301 crdlhpgpvs llhwsgsgkp wirldskrpc pldalwtpyd lyrhsh

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Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations of any two or more of said steps or features.

The amino acids which occur in the various amino acid sequences referred to in the specification have their usual three- and one-letter abbreviations routinely used in the art: A, Ala, Alanine; C, Cys, Cysteine; D, Asp, Aspartic Acid; E, Glu, Glutamic Acid; F, Phe, Phenylalanine; G, Gly, Glycine; H, His, Histidine; I, Ile, Isoleucine; K, Lys, Lysine; L, Leu, Leucine; M, Met, Methionine; N, Asn, Asparagine; P, Pro, Proline; Q, Gln, Glutamine; R, Arg, Arginine; S, Ser, Serine; T, Thr, Threonine; V, Val, Valine; W, Try, Tryptophan; Y, Tyr, Tyrosine.

A protein is considered an isolated protein if it is a protein isolated from the plant, or from a host cell in which it is recombinantly produced. It can be purified or it can simply be free of other proteins and biological materials with which it is associated in nature.

An isolated nucleic acid is a nucleic acid the structure of which is not identical to that of any naturally occurring nucleic acid or to that of any fragment of a naturally occurring genomic nucleic acid spanning more than three separate genes. The term therefore covers, for example, (a) a DNA which has the sequence

of part of a naturally occurring genomic DNA molecule but is not flanked by both of the coding or noncoding sequences that flank that part of the molecule in the genome of the organism in which it naturally occurs; (b) a nucleic acid incorporated into a vector or into the genomic DNA of a prokaryote or eukaryote in a manner such that the resulting molecule is not identical to any naturally occurring vector or genomic DNA; (c) a separate molecule such as a cDNA, a genomic fragment, a fragment produced by polymerase chain reaction (PCR), or a restriction fragment; and (d) a recombinant nucleotide sequence that is part of a hybrid gene, i.e., a gene encoding a fusion protein. Specifically excluded from this definition are nucleic acids present in mixtures of (i) DNA molecules, (ii) transformed or transfected cells, and (iii) cell clones, e.g., as these occur in a DNA library such as a cDNA or genomic DNA library.

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As used herein expression directed by a particular sequence is the transcription of an associated downstream sequence. If appropriate and desired for the associated sequence, there the term expression also encompasses translation (protein synthesis) of the transcribed RNA. When expression of a sequence of interest is "up-regulated," the expression is increased. With reference to up-regulation of expression of a sequence of interest operably linked to a transcription regulatory sequence, expression is increased.

In the present context, a promoter is a DNA region which includes sequences sufficient to cause transcription of an associated (downstream) sequence. The promoter may be regulated, i.e., not constitutively acting to cause transcription of the associated sequence. If inducible, there are sequences present which mediate regulation of expression so that the associated sequence is transcribed only when an inducer molecule is present in the medium in or on which the organism is cultivated. In the present context, a transcription regulatory sequence includes a promoter sequence and can further include cis-active sequences for regulated expression of an associated sequence in response to environmental signals.

One DNA portion or sequence is downstream of second DNA portion or sequence when it is located 3' of the second sequence. One DNA portion or

sequence is upstream of a second DNA portion or sequence when it is located 5' of that sequence.

One DNA molecule or sequence and another are heterologous to another if the two are not derived from the same ultimate natural source. The sequences may be natural sequences, or at least one sequence can be designed by man, as in the case of a multiple cloning site region. The two sequences can be derived from two different species or one sequence can be produced by chemical synthesis provided that the nucleotide sequence of the synthesized portion was not derived from the same organism as the other sequence.

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An isolated or substantially pure nucleic acid molecule or polynucleotide is a polynucleotide which is substantially separated from other polynucleotide sequences which naturally accompany a native transcription regulatory sequence. The term embraces a polynucleotide sequence which has been removed from its naturally occurring environment, and includes recombinant or cloned DNA isolates, chemically synthesized analogues and analogues biologically synthesized by heterologous systems.

A polynucleotide is said to encode a polypeptide if, in its native state or when manipulated by methods known to those skilled in the art, it can be transcribed and/or translated to produce the polypeptide or a fragment thereof. The anti-sense strand of such a polynucleotide is also said to encode the sequence.

A nucleotide sequence is operably linked when it is placed into a functional relationship with another nucleotide sequence. For instance, a promoter is operably linked to a coding sequence if the promoter effects its transcription or expression. Generally, operably linked means that the sequences being linked are contiguous and, where necessary to join two protein coding regions, contiguous and in reading frame. However, it is well known that certain genetic elements, such as enhancers, may be operably linked even at a distance, i.e., even if not contiguous.

The term recombinant polynucleotide refers to a polynucleotide which is made by the combination of two otherwise separated segments of sequence accomplished by the artificial manipulation of isolated segments of polynucleotides by genetic engineering techniques or by chemical synthesis. In so doing one may join together polynucleotide segments of desired functions to generate a desired combination of functions.

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Polynucleotide probes include an isolated polynucleotide attached to a label or reporter molecule and may be used to identify and isolate other sequences, for example, those from other species or other strains. Probes comprising synthetic oligonucleotides or other polynucleotides may be derived from naturally occurring or recombinant single or double stranded nucleic acids or be chemically synthesized. Polynucleotide probes may be labeled by any of the methods known in the art, e.g., random hexamer labeling, nick translation, or the Klenow fill-in reaction.

Large amounts of the polynucleotides may be produced by replication in a suitable host cell. Natural or synthetic DNA fragments coding for a protein of interest are incorporated into recombinant polynucleotide constructs, typically DNA constructs, capable of introduction into and replication in a prokaryotic or eukaryotic cell. Usually the construct is suitable for replication in a unicellular host, such as *A. pullulans* or a bacterium, but a multicellular eukaryotic host may also be appropriate, with or without integration within the genome of the host cell. Commonly used prokaryotic hosts include strains of *Escherichia coli*, although other prokaryotes, such as *Bacillus subtilis* or a pseudomonad, may also be used. Eukaryotic host cells include yeast, filamentous fungi, plant, insect, amphibian, mammalian and avian species. Such factors as ease of manipulation, ability to appropriately glycosylate expressed proteins, degree and control of protein expression, ease of purification of expressed proteins away from cellular contaminants or other factors influence the choice of the host cell.

The polynucleotides may also be produced by chemical synthesis, e.g., by the phosphoramidite method described by Beaucage and Caruthers (1981) *Tetra. Letts.*, **22**: 1859-1862 or the triester method according to Matteuci *et al.* (1981) *J.* 

Am. Chem. Soc., 103:3185, and may be performed on commercial automated oligonucleotide synthesizers. A double-stranded fragment may be obtained from the single stranded product of chemical synthesis either by synthesizing the complementary strand and annealing the strand together under appropriate conditions or by adding the complementary strand using DNA polymerase with an appropriate primer sequence.

DNA constructs prepared for introduction into a prokaryotic or eukaryotic host will typically comprise a replication system (i.e. vector) recognized by the host, including the intended DNA fragment encoding the desired polypeptide, and will preferably also include transcription and translational initiation regulatory sequences operably linked to the polypeptide-encoding segment. Expression systems (expression vectors) may include, for example, an origin of replication or autonomously replicating sequence (ARS) and expression control sequences, a promoter, an enhancer and necessary processing information sites, such as ribosome-binding sites, RNA splice sites, polyadenylation sites, transcriptional terminator sequences, and mRNA stabilizing sequences. Signal peptides may also be included where appropriate from secreted polypeptides of the same or related species, which allow the protein to cross and/or lodge in cell membranes or be secreted from the cell.

An appropriate promoter and other necessary vector sequences will be selected so as to be functional in the host. Examples of workable combinations of cell lines and expression vectors are described in Sambrook *et al.* (1989) vide infra; Ausubel *et al.* (Eds.) (1995) *Current Protocols in Molecular Biology*, Greene Publishing and Wiley Interscience, New York; and Metzger *et al.* (1988) *Nature*, 334: 31-36. Many useful vectors for expression in bacteria, yeast, fungal, mammalian, insect, plant or other cells are well known in the art and may be obtained such vendors as Stratagene, New England Biolabs, Promega Biotech, and others. In addition, the construct may be joined to an amplifiable gene (e.g., DHFR) so that multiple copies of the gene may be made. For appropriate enhancer and other expression control sequences, see also *Enhancers and Eukaryotic Gene Expression*, Cold Spring Harbor Press, N.Y. (1983). While such expression vectors

may replicate autonomously, they may less preferably replicate by being inserted into the genome of the host cell.

Expression and cloning vectors will likely contain a selectable marker, that is, a gene encoding a protein necessary for the survival or growth of a host cell transformed with the vector. Although such a marker gene may be carried on another polynucleotide sequence co-introduced into the host cell, it is most often contained on the cloning vector. Only those host cells into which the marker gene has been introduced will survive and/or grow under selective conditions. Typical selection genes encode proteins that (a) confer resistance to antibiotics or other toxic substances, e.g., ampicillin, neomycin, methotrexate, etc.; (b) complement auxotrophic deficiencies; or (c) supply critical nutrients not available from complex media. The choice of the proper selectable marker will depend on the host cell; appropriate markers for different hosts are known in the art.

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Recombinant host cells, in the present context, are those which have been genetically modified to contain an isolated DNA molecule of the instant invention. The DNA can be introduced by any means known to the art which is appropriate for the particular type of cell, including without limitation, transformation, lipofection or electroporation.

It is recognized by those skilled in the art that the DNA sequences may vary due to the degeneracy of the genetic code and codon usage. All DNA sequences which code for the polypeptide or protein of interest are included in this invention.

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Additionally, it will be recognized by those skilled in the art that allelic variations may occur in the DNA sequences which will not significantly change activity of the amino acid sequences of the peptides which the DNA sequences encode. All such equivalent DNA sequences are included within the scope of this invention and the definition of the regulated promoter region. The skilled artisan will understand that the sequence of the exemplified sequence can be used to identify and isolate additional, nonexemplified nucleotide sequences which are functionally equivalent to the sequences given.

Mutational, insertional, and deletional variants of the disclosed nucleotide sequences can be readily prepared by methods which are well known to those skilled in the art. These variants can be used in the same manner as the exemplified primer sequences so long as the variants have substantial sequence homology with the original sequence. As used herein, substantial sequence homology refers to homology which is sufficient to enable the variant polynucleotide to function in the same capacity as the polynucleotide from which the probe was derived. Preferably, this homology is greater than 80%, more preferably, this homology is greater than 85%, even more preferably this homology is greater than 90%, and most preferably, this homology is greater than 95%. The degree of homology or identity needed for the variant to function in its intended capacity depends upon the intended use of the sequence. It is well within the skill of a person trained in this art to make mutational, insertional, and deletional mutations which are equivalent in function or are designed to improve the function of the sequence or otherwise provide a methodological advantage.

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Polymerase Chain Reaction (PCR) is a repetitive, enzymatic, primed synthesis of a nucleic acid sequence. This procedure is well known and commonly used by those skilled in this art [see Mullis, U.S. Patent Nos. 4,683,195, 4,683,202, and 4,800,159; Saiki et al. (1985) Science 230:1350-1354]. PCR is based on the enzymatic amplification of a DNA fragment of interest that is flanked by two oligonucleotide primers that hybridize to opposite strands of the target sequence. The primers are oriented with the 3' ends pointing towards each other. Repeated cycles of heat denaturation of the template, annealing of the primers to their complementary sequences, and extension of the annealed primers with a DNA polymerase result in the amplification of the segment defined by the 5' ends of the PCR primers. Since the extension product of each primer can serve as a template for the other primer, each cycle essentially doubles the amount of DNA template produced in the previous cycle. This results in the exponential accumulation of the specific target fragment, up to several million-fold in a few hours. By using a thermostable DNA polymerase such as the Taq polymerase, which is isolated from the thermophilic bacterium Thermus aquaticus, the amplification process can be completely automated. Other enzymes which can be used are known to those skilled in the art.

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It is well known in the art that the polynucleotide sequences of the present invention can be truncated and/or mutated such that certain of the resulting fragments and/or mutants of the original full-length sequence can retain the desired characteristics of the full-length sequence. A wide variety of restriction enzymes which are suitable for generating fragments from larger nucleic acid molecules are In addition, it is well known that Bal31 exonuclease can be well known. conveniently used for time-controlled limited digestion of DNA. See, for example, Maniatis (1982) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, New York, pages 135-139, incorporated herein by reference. See also Wei et al. (1983 J. Biol. Chem. 258:13006-13512. By use of Bal31 exonuclease (commonly referred to as "erase-a-base" procedures), the ordinarily skilled artisan can remove nucleotides from either or both ends of the subject nucleic acids to generate a wide spectrum of fragments which are functionally equivalent to the subject nucleotide sequences. One of ordinary skill in the art can, in this manner, generate hundreds of fragments of controlled, varying lengths from locations all along the original molecule. The ordinarily skilled artisan can routinely test or screen the generated fragments for their characteristics and determine the utility of the fragments as taught herein. It is also well known that the mutant sequences of the full length sequence, or fragments thereof, can be easily produced with site directed mutagenesis. See, for example, Larionov, O.A. and Nikiforov, V.G. (1982) Genetika 18(3):349-59; Shortle, D, DiMaio, D., and Nathans, D. (1981) Annu. Rev. Genet. 15:265-94; both incorporated herein by reference. The skilled artisan can routinely produce deletion-, insertion-, or substitution-type mutations and identify those resulting mutants which contain the desired characteristics of the full length wild-type sequence, or fragments thereof, i.e., those which retain promoter activity and also provide transcription of downstream sequence.

Following the teachings herein and using knowledge and techniques well known in the art, the skilled worker will be able to make a large number of operative embodiments having equivalent DNA sequences to those listed herein without the expense of undue experimentation.

As used herein percent sequence identity of two nucleic acids is determined using the algorithm of Karlin and Altschul (1990) *Proc. Natl. Acad. Sci. USA* 

87:2264-2268, modified as in Karlin and Altschul (1993) *Proc. Natl. Acad. Sci. USA* 90:5873-5877. Such an algorithm is incorporated into the NBLAST and XBLAST programs of Altschul *et al.* (1990) *J. Mol. Biol.* 215:402-410. BLAST nucleotide searches are performed with the NBLAST program, score = 100, wordlength = 12, to obtain nucleotide sequences with the desired percent sequence identity. To obtain gapped alignments for comparison purposes, Gapped BLAST is used as described in Altschul *et al.* (1997) *Nucl. Acids. Res.* 25:3389-3402. When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (NBLAST and XBLAST) are used. See, for example, the National Center for Biotechnology Information website on the internet.

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Techniques and agents for introducing and selecting for the presence of heterologous DNA in plant cells and/or tissue are well-known. Genetic markers allowing for the selection of heterologous DNA in plant cells are well-known, e.g., genes carrying resistance to an antibiotic such as kanamycin, hygromycin, The marker allows for selection of successfully gentamicin, or bleomycin. transformed plant cells growing in the medium containing the appropriate antibiotic because they will carry the corresponding resistance gene. In most cases the heterologous DNA which is inserted into plant cells contains a gene which encodes a selectable marker such as an antibiotic resistance marker, but this is not mandatory. An exemplary drug resistance marker is the gene whose expression results in kanamycin resistance, i.e., the chimeric gene containing nopaline synthetase promoter, Tn5 neomycin phosphotransferase II and nopaline synthetase 3' non-translated region described by Rogers et al., Methods for Plant Molecular Biology, A. Weissbach and H. Weissbach, eds., Academic Press, Inc., San Diego, CA (1988).

Techniques for genetically engineering plant cells and/or tissue with an expression cassette comprising an inducible promoter or chimeric promoter fused to a heterologous coding sequence, including possibly an antisense DNA construct and/or a DNA construct designed to elicit double-stranded RNA-mediated gene silencing, followed by a transcription termination sequence are to be introduced into the plant cell or tissue by *Agrobacterium*- mediated transformation, electroporation, microinjection, particle bombardment or other techniques known to the art. The

expression cassette advantageously further contains a marker allowing selection of the heterologous DNA in the plant cell, e.g., a gene carrying resistance to an antibiotic such as kanamycin, hygromycin, gentamicin, or bleomycin.

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A DNA construct carrying a plant-expressible gene or other DNA of interest can be inserted into the genome of a plant by any suitable method. Such methods may involve, for example, the use of liposomes, electroporation, diffusion, particle bombardment, microinjection, gene gun, chemicals that increase free DNA uptake, e.g., calcium phosphate coprecipitation, viral vectors, and other techniques practiced in the art. Suitable plant transformation vectors include those derived from a Ti plasmid of *Agrobacterium tumefaciens*, such as those disclosed by Herrera-Estrella (1983), Bevan (1983), Klee (1985) and EPO publication 120,516 (Schilperoort *et al.*). In addition to plant transformation vectors derived from the Ti or root-inducing (Ri) plasmids of *Agrobacterium*, alternative methods can be used to insert the DNA constructs of this invention into plant cells.

The choice of vector in which the DNA of interest is operatively linked depends directly, as is well known in the art, on the functional properties desired, e.g., replication, protein expression, and the host cell to be transformed, these being limitations inherent in the art of constructing recombinant DNA molecules. The vector desirably includes a prokaryotic replicon, i.e., a DNA sequence having the ability to direct autonomous replication and maintenance of the recombinant DNA molecule extra-chromosomally when introduced into a prokaryotic host cell, such as a bacterial host cell. Such replicons are well known in the art. In addition, preferred embodiments that include a prokaryotic replicon also include a gene whose expression confers a selective advantage, such as a drug resistance, to the bacterial host cell when introduced into those transformed cells.

Typical bacterial drug resistance genes are those that confer resistance to ampicillin or tetracycline, among other selective agents. The neomycin phosphotransferase gene has the advantage that it is expressed in eukaryotic as well as prokaryotic cells.

Those vectors that include a prokaryotic replicon also typically include convenient restriction sites for insertion of a recombinant DNA molecule of the present invention. Typical of such vector plasmids are pUC8, pUC9, pBR322, and pBR329 available from BioRad Laboratories (Richmond, CA) and pPL, pK and K223 available from Pharmacia (Piscataway, NJ), and pBLUESCRIPT and pBS available from Stratagene (La Jolla, CA). A vector of the present invention may also be a Lambda phage vector including those Lambda vectors described in Molecular Cloning: A Laboratory Manual, Second Edition, Maniatis *et al.*, eds., Cold Spring Harbor Press (1989) and the Lambda ZAP vectors available from Stratagene (La Jolla, CA). Other exemplary vectors include pCMU [Nilsson *et al.* (1989) *Cell* 58:707]. Other appropriate vectors may also be synthesized, according to known methods; for example, vectors pCMU/Kb and pCMUII used in various applications herein are modifications of pCMUIV [Nilsson, (1989) supra].

Typical expression vectors capable of expressing a recombinant nucleic acid sequence in plant cells and capable of directing stable integration within the host plant cell include vectors derived from the tumor-inducing (Ti) plasmid of *Agrobacterium tumefaciens* described by Rogers *et al.* (1987) *Meth. in Enzymol.* **153**:253-277, and several other expression vector systems known to function in plants. See for example, Verma *et al.*, No. WO87/00551; Cocking and Davey (1987) *Science* **236**:1259-1262.

A transgenic plant can be produced by any means known to the art, including but not limited to *Agrobacterium tumefaciens*-mediated DNA transfer, preferably with a disarmed T-DNA vector, electroporation, direct DNA transfer, and particle bombardment [See Davey *et al.* (1989) *Plant Mol. Biol.* 13:275; Walden and Schell (1990) *Eur. J. Biochem.* 192:563; Joersbo and Burnstedt (1991) *Physiol. Plant.* 81:256; Potrykus (1991) *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 42:205; Gasser and Fraley (1989) *Science* 244:1293; Leemans (1993) *Bio/Technology* 11:522; Beck *et al.* (1993) *Bio/Technology* 11:1524; Koziel *et al.* (1993) *Bio/Technology* 11:1533 and Gelvin, S.B. (1999) *Curr. Opin. Biotech.* 9:227-232]. Techniques are well-known to the art for the introduction of DNA into monocots as well as dicots, as are the techniques for culturing such plant tissues and regenerating those tissues.

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Standard techniques for cloning, DNA isolation, amplification and purification, for enzymatic reactions involving DNA ligase, DNA polymerase, restriction endonucleases and the like, and various separation techniques are those known and commonly employed by those skilled in the art. A number of standard techniques are described in Sambrook et al. (1989) Molecular Cloning, Second Edition, Cold Spring Harbor Laboratory, Plainview, New York; Maniatis et al. (1982) Molecular Cloning, Cold Spring Harbor Laboratory, Plainview, New York; Wu (ed.) (1993) Meth. Enzymol. 218, Part I; Wu (ed.) (1979) Meth. Enzymol. 68; Wu et al. (eds.) (1983) Meth. Enzymol. 100 and 101; Grossman and Moldave (eds.) Meth. Enzymol. 65; Miller (ed.) (1972) Experiments in Molecular Genetics, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York; Old and Primrose (1981) Principles of Gene Manipulation, University of California Press, Berkley; Schleif and Wensink (1982) Practical Methods in Molecular Biology; Glover (ed.) (1985) DNA Cloning Vol. I and II, IRL Press, Oxford, UK; Hames and Higgins (eds.) (1985) Nucleic Acid Hybridization, IRL Press, Oxford, UK; Setlow and Hollaender (1979) Genetic Engineering: Principles and Methods, Vols. 1-4, Plenum Press, New York; and Ausubel et al. (1992) Current Protocols in Molecular Biology, Greene/Wiley, New York, NY. Abbreviations and nomenclature, where employed, are deemed standard in the field and commonly used in professional journals such as those cited herein.

All references cited in the present application are incorporated in their entirety herein by reference to the extent not inconsistent herewith.

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#### **CLAIMS**

#### We claim:

1. An isolated nucleic acid encoding a polypeptide or a fragment thereof having galacturonosyltransferase (GalAT) activity.

- 2. The nucleic acid of claim 1 wherein the polypeptide or the fragment has approximately 50% amino acid sequence similarity with the corresponding sequence as set forth in SEQ ID NO: 2.
- 3. The nucleic acid of claim 2 wherein the amino acid molecule is selected from the group consisting of the sequences as set forth in SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, and 50.
- 4. The nucleic acid of claim 3 wherein the polypeptide comprises the amino acid sequence as set forth in SEQ ID NO: 2.
- 5. The nucleic acid of claim 4 wherein the polypeptide is encoded by the nucleic acid sequence as set forth in SEQ ID NO: 1.
- 6. An isolated polypeptide or a fragment thereof having galacturonosyltransferase GalAT activity wherein the polypeptide or the fragment has approximately 50% amino acid sequence similarity with the corresponding amino acid sequence as shown in SEQ ID NO: 2.
- 7. The polypeptide or the fragment of claim 6 which comprises the amino acid sequence selected from the group consisting of the sequences as set forth in SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, and 50, or the corresponding sequence thereto.
- 8. The polypeptide or the fragment of claim 7 which comprises the amino acid sequence as set forth in SEQ ID NO: 2 or the corresponding sequence

thereto.

9. The polypeptide or the fragment of claim 7 wherein the amino acid sequence is encoded by the nucleic acid as set forth in SEQ ID NO: 1.

- 10. An antibody which specifically recognizes the polypeptide or the fragment of claims 7 or 8.
- 11. An expression vector comprising in operable linkage the nucleic acid according to any one of claims 1, 2, 3 or 5 and a plant-expressible promoter.
- 12. The expression vector of claim 11 wherein said promoter is heterologous to said nucleic acid.
- 13. A transgenic plant which has been transformed with the expression vector of claims 11 or 12.
- 14. A transgenic plant having modified pectin.
- 15. A transgenic plant having altered GalAT activity wherein the altered activity is due to a mutation in the *GALAT* gene.
- 16. Progeny of the transgenic plant of claims 13, 14 or 15.
- 17. Modified pectin isolated from the transgenic plant of claims 14 or 15.
- 18. A product comprising the modified pectin of claim 17.
- 19. A method of generating a plant with altered GalAT activity by mutating the *GALAT* gene.

20. A method of preparing a polymer comprising a galacturonic acid and a polymer with a GALAT protein under conditions suitable to form at least one covalent linkage between the galacturonic acid and the polymer.

- 21. The method of claim 20 wherein said polymer is selected from the group consisting of homogalacturonan, rhamnogalacturonan I, rhamnogalacturonan II, xylogalacturonan, apiogalacturonan or other galacturonic containing polymer.
- 22. The method of claim 21, wherein said polymer is homogalacturonan.
- 23. The method of claims 20 or 21 wherein the GALAT protein comprises the amino acid sequence as set forth in SEQ ID NO: 2 or a fragment thereof having GalAT activity.

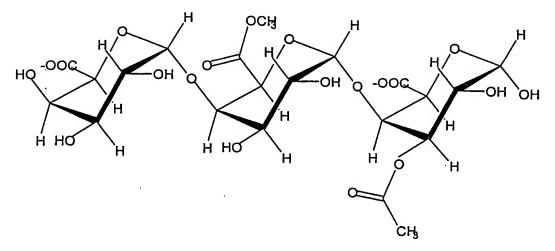


FIG. 1

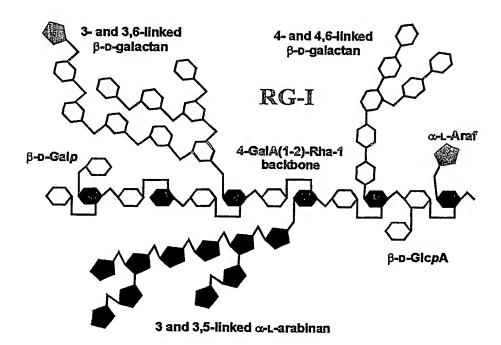


FIG. 2

## **RG-II**

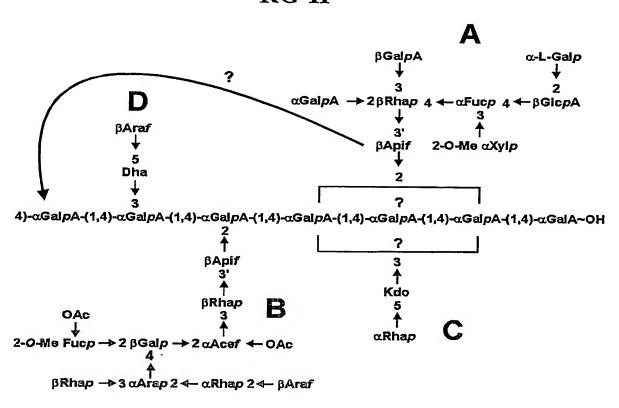
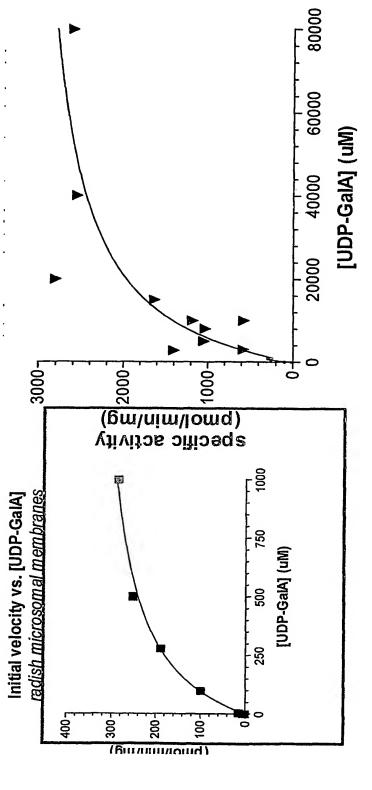


FIG. 3



16.4

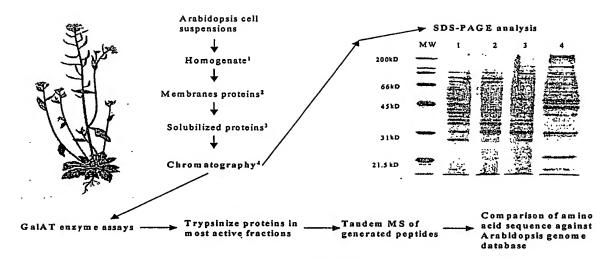


FIG. 5

5/8

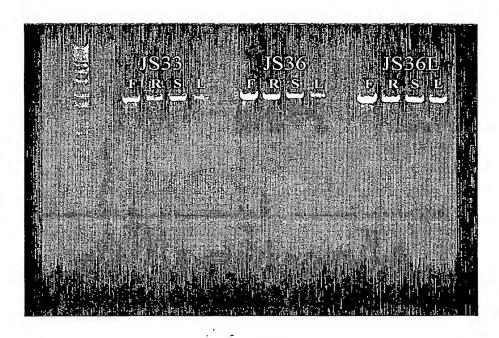


FIG. 6A

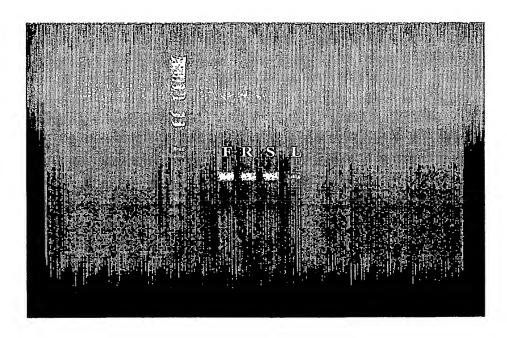
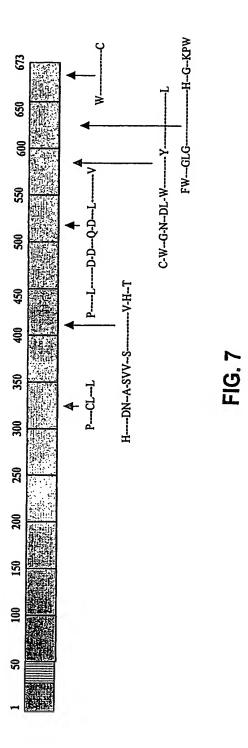


FIG. 6B



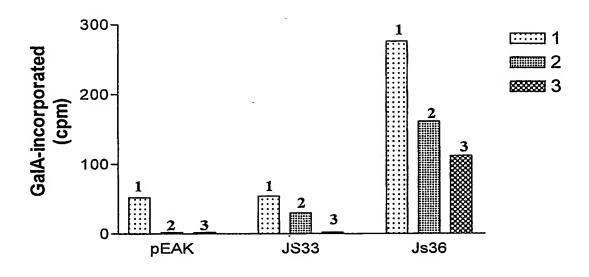


FIG. 8

## Arabidopsis GalAT Superfamily

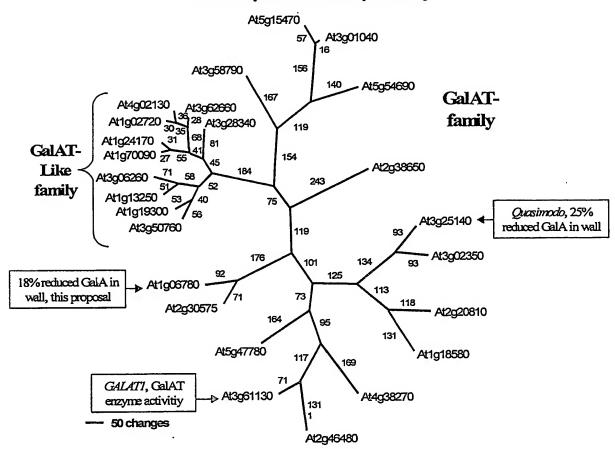


FIG. 9

# 14-03 WO.sequence listing.txt SEQUENCE LISTING

University of Georgia Research Foundation, Inc <110> Mohnen, Debra Hahn, Michael G. Kolli, Venkata S.K. Doong, Ron L. Sterling, Jason D. <120> Galacturonosyltransferases, nucleic acids encoding same, and uses therefor 14-03 WO <130> <140> Not assigned <141> 2004-02-05 us 60/445,539 2003-02-06 <150> <151> <160> 50 <170> PatentIn version 3.2 <210> 2022 <211> <212> DNA Arabidopsis thaliana <213> <400> atggcgctaa agcgagggct atctggagtt aaccggatta gaggaagtgg tggtggatct 60 cgatctgtgc ttgtgcttct catatttttc tgtgtttttg cacctctttg cttctttgtt 120 ggccgaggag tgtatatcga ttcctcaaat gattattcaa ttgtttctgt gaagcagaat 180 cttgactgga gagaacgttt agcaatgcaa tctgttagat ctcttttctc gaaagagata 240 ctagatgtta tagcaaccag cacagctgat ttgggtcctc ttagccttga ttcttttaag 300 aaaaacaatt tgtctgcatc atggcgggga accggagtag acccctcctt tagacattct 360 gagaatccag caactcctga tgtcaaatct aataacctga atgaaaaacg tgacagcatt 420 tcaaaagata gtatccatca gaaagttgag acacctacaa agattcacag aaggcaacta 480 540 agagagaaaa ggcgtgagat gcgggcaaat gagttagttc agcacaatga tgacacgatt ttgaaactcg aaaatgctgc cattgaacgc tctaagtctg ttgattctgc agtccttggt 600 aaatacagta tttggagaag agaaaatgag aatgacaact ctgattcaaa tatacgcttg 660 720 atgcgggatc aagtaataat ggctagagtc tatagtggga ttgcaaaatt gaaaaacaag 780 aacgatttgt tacaagaact ccaggcccga cttaaggaca gccaacgggt tttgggggaa gcaacatctg atgctgatct tcctcggagt gcgcatgaga aactcagagc catgggtcaa 840 gtcttggcta aagctaagat gcagttatat gactgcaagc tggttactgg aaagctgaga 900 gcaatgcttc agactgccga cgaacaagtg aggagcttaa agaagcagag tactttctg 960 1020 gctcagttag cagcaaaaac cattccaaat cctatccatt gcctatcaat gcgcttgact Page 1

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PCT/US2004/003545 WO 2004/072250

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Arabidopsis thaliana

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14-03 WO.sequence listing.txt

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Pro Gly Lys Ala Thr Ile His Val Glu Asn Val Asp Glu Phe Lys Trp 420 425 430

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Ser Leu Ser Ala Glu Phe Arg Val Ser Phe Pro Ser Gly Asp Leu Leu 405 410

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Leu Asp Met Glu Gly Lys Val Asn Gly Ala Val Lys Ser Cys Thr Val 465 470 480

Arg Leu Gly Gln Leu Arg Ser Leu Lys Arg Gly Asn Phe Asp Thr Asn 485 490 495

Ala Cys Leu Trp Met Ser Gly Leu Asn Val Val Asp Leu Ala Arg Trp 500 505

Arg Ala Leu Gly Val Ser Glu Thr Tyr Gln Lys Tyr Tyr Lys Glu Met 515 525

Ser Ser Gly Asp Glu Ser Ser Glu Ala Ile Ala Leu Gln Ala Ser Leu 530 540

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Gly Ile Pro Asn Tyr Lys Asn Tyr Trp Arg Arg His Leu Ser Arg Glu 595 600 605 Page 8

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Leu Thr Gln Gln Thr Ser Glu Lys Val Asp Glu Gln Pro Glu Pro Asn 130 135 140

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14-03 WO.sequence listing.txt

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Gln Asn Ile Thr Glu Val Tyr His Arg Trp Gln Asp Leu Asn Gln Asp 515 520 525

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14-03 WO.sequence listing.txt

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Asp Gly Glu Gly Leu Lys Gly Pro Arg Leu Ile Leu Phe Lys Asp Gly 65 70 75 80

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Arg Glu Glu Gln Val Ile Val Ser Gln Lys Met Thr Val Ser Ser Asp 100 105

Glu Lys Gly Gln Ile Leu Pro Thr Val Asn Gln Leu Ala Asn Lys Thr 115 120 125

Asp Phe Lys Pro Pro Leu Ser Lys Gly Glu Lys Asn Thr Arg Val Gln 130 140

Pro Asp Arg Ala Thr Asp Val Lys Thr Lys Glu Ile Arg Asp Lys Ile 145 150 155 160

Ile Gln Ala Lys Ala Tyr Leu Asn Phe Ala Pro Pro Gly Ser Asn Ser 165 170 175

Gln Val Val Lys Glu Leu Arg Gly Arg Leu Lys Glu Leu Glu Arg Ser 180 185 190

Val Gly Asp Ala Thr Lys Asp Lys Asp Leu Ser Lys Gly Ala Leu Arg 195 200 205

Arg Val Lys Pro Met Glu Asn Val Leu Tyr Lys Ala Ser Arg Val Phe 210 215 220

Asn Asn Cys Pro Ala Ile Ala Thr Lys Leu Arg Ala Met Asn Tyr Asn 225 230 235

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Gln Leu Ala Ala Arg Thr Thr Pro Lys Gly Leu His Cys Leu Ser Met 260 265 270 Page 14

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485 490 495 Asn Leu Gly Thr Lys Arg Pro Leu Trp Lys Ala Gly Ser Leu Pro Ile 500 505 Gly Trp Leu Thr Phe Tyr Arg Gln Thr Leu Ala Leu Asp Lys Arg Trp Page 15

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415
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Arg Glu Arg Thr Leu Trp Lys Leu Gly Thr Leu Pro Pro Gly Leu Leu 450 460

Ser Phe Tyr Gly Leu Thr Glu Pro Leu Asp Arg Arg Trp His Val Leu 465 470 475 480

Gly Leu Gly Tyr Asp Val Asn Ile Asp Asn Arg Leu Ile Glu Thr Ala 485 490 495

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<sup>&</sup>lt;210> 11

<sup>&</sup>lt;211> 1611 <212> DNA

<sup>&</sup>lt;213> Arabidopsis thaliana

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<sup>536</sup> 

<sup>&</sup>lt;213> Arabidopsis thaliana

14-03 WO.sequence listing.txt
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14-03 WO.sequence listing.txt

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Arg Lys Arg Asn Val Thr Gly Ile Tyr His Tyr Trp Gln Glu Lys Asn 435 440 445

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Gly Leu Gly Tyr Thr Asn Val Asp Ala Arg Val Ile Glu Lys Gly Ala 485 490 495

Val Leu His Phe Asn Gly Asn Leu Lys Pro Trp Leu Lys Ile Gly Ile 500 505 510

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<212> DNA

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Met Asn Gln Val Arg Arg Trp Gln Arg Ile Leu Ile Leu Ser Leu Leu Page 23

<sup>&</sup>lt;210> 14 <211> 610 <212> PRT <213> Arabidopsis thaliana

<sup>&</sup>lt;400> 14

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165 170 175 Glu Ile Arg Asp Lys Ile Ile Gln Ala Lys Ala Tyr Leu Asn Leu Ala 180 185 190 Leu Pro Gly Asn Asn Ser Gln Ile Val Lys Glu Leu Arg Val Arg Thr 195 200 205

Pro Lys Ser Ser Pro Asn Arg Leu Lys Ala Met Glu Val Ala Leu Tyr 235

Lys Val Ser Arg Ala Phe His Asn Cys Pro Ala Ile Ala Thr Lys Leu 245

Lys Glu Leu Glu Arg Ala Thr Gly Asp Thr Thr Lys Asp Lys Tyr Leu 210 215 220

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PCT/US2004/003545 WO 2004/072250

14-03 WO.sequence listing.txt Gln Ala Met Thr Tyr Lys Thr Glu Glu Gln Ala Arg Ala Gln Lys Lys 260 265 270 Gln Ala Ala Tyr Leu Met Gln Leu Ala Ala Arg Thr Thr Pro Lys Gly 285 Leu His Cys Leu Ser Met Arg Leu Thr Thr Glu Tyr Phe Thr Leu Asp 290 295 300 His Glu Lys Arg Gln Leu Leu Gln Gln Ser Tyr Asn Asp Pro Asp Leu 305 310 315 Tyr His Tyr Val Val Phe Ser Asp Asn Val Leu Ala Ser Ser Val Val 325 330 335 Val Asn Ser Thr Ile Ser Ser Ser Lys Glu Pro Asp Lys Ile Val Phe 340 350 His Val Val Thr Asp Ser Leu Asn Tyr Pro Ala Ile Ser Met Trp Phe 355 360 Leu Leu Asn Pro Ser Gly Arg Ala Ser Ile Gln Ile Leu Asn Ile Asp 370 380 Glu Met Asn Val Leu Pro Leu Tyr His Ala Glu Leu Leu Met Lys Gln 385 390 395 400 Asn Ser Ser Asp Pro Arg Ile Ile Ser Ala Leu Asn His Ala Arg Phe 405 410 415 Tyr Leu Pro Asp Ile Phe Pro Gly Leu Asn Lys Ile Val Leu Phe Asp 420 425 430 His Asp Val Val Gln Arg Asp Leu Thr Arg Leu Trp Ser Leu Asp 445 Met Thr Gly Lys Val Val Gly Ala Val Glu Thr Cys Leu Glu Gly Asp 450 455 460 Pro Ser Tyr Arg Ser Met Asp Ser Phe Ile Asn Phe Ser Asp Ala Trp 465 470 480 Val Ser Gln Lys Phe Asp Pro Lys Ala Cys Thr Trp Ala Phe Gly Met 485 490 495 Asn Leu Phe Asp Leu Glu Glu Trp Arg Arg Gln Glu Leu Thr Ser Val Page 25

14-03 WO.sequence listing.txt

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<213> Arabidopsis thaliana

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Ala Asn Glu Leu Met Asn Asp Asp Ser Leu Gln Lys Leu Glu Thr Ala 50 60

Ala Met Ala Arg Ser Arg Ser Val Asp Ser Ala Pro Leu Gly Asn Tyr 65 70 75 80

Thr Ile Trp Lys Asn Glu Tyr Arg Arg Gly Lys Ser Phe Glu Asp Met 85 90 95

Leu Arg Leu Met Gln Asp Gln Ile Ile Met Ala Arg Val Tyr Ser Gly 100 105 110

Leu Ala Lys Phe Thr Asn Asn Leu Ala Leu His Gln Glu Ile Glu Thr Page 27 14-03 WO.sequence listing.txt 115 120 125

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14-03 WO.sequence listing.txt
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540

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18 533

Arabidopsis thaliana

Met Gln Leu His Ile Ser Pro Ser Met Arg Ser Ile Thr Ile Ser Ser 10 15

Ser Asn Glu Phe Ile Asp Leu Met Lys Ile Lys Val Ala Ala Arg His 20 25 30

Ile Ser Tyr Arg Thr Leu Phe His Thr Ile Leu Ile Leu Ala Phe Leu 35 40 45

Leu Pro Phe Val Phe Ile Leu Thr Ala Val Val Thr Leu Glu Gly Val 50 60

Asn Lys Cys Ser Ser Phe Asp Cys Phe Gly Arg Arg Leu Gly Pro Arg 65 Page 30

### 14-03 WO.sequence listing.txt

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14-03 WO.sequence listing.txt 325 330 335

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Leu Ile Ala Lys His Leu Asp Pro Glu Glu Cys Ala Trp Ala Tyr Gly 405 410 415

Met Asn Ile Phe Asp Leu Arg Thr Trp Arg Lys Thr Asn Ile Arg Glu 420 425 430

Thr Tyr His Ser Trp Leu Lys Glu Asn Leu Lys Ser Asn Leu Thr Met 435 440 445

Trp Lys Leu Gly Thr Leu Pro Pro Ala Leu Ile Ala Phe Lys Gly His 450 460

Val Gln Pro Ile Asp Ser Ser Trp His Met Leu Gly Leu Gly Tyr Gln 465 470 475 480

Ser Lys Thr Asn Leu Glu Asn Ala Lys Lys Ala Ala Val Ile His Tyr 485 490 495

Asn Gly Gln Ser Lys Pro Trp Leu Glu Ile Gly Phe Glu His Leu Arg 500 505

Pro Phe Trp Thr Lys Tyr Val Asn Tyr Ser Asn Asp Phe Ile Lys Asn 515 520 525

Cys His Ile Leu Glu 530

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Ala Tyr Met Gln Arg Thr Phe Leu Ala Leu Gln Ser Asp Pro Leu Lys 65 70 75 80

Thr Arg Leu Asp Leu Ile His Lys Gln Ala Ile Asp His Leu Thr Leu 85 90 95

Val Asn Ala Tyr Ala Ala Tyr Ala Arg Lys Leu Lys Leu Asp Ala Ser 100 105 110

Lys Gln Leu Lys Leu Phe Glu Asp Leu Ala Ile Asn Phe Ser Asp Leu 115 120 125

Gln Ser Lys Pro Gly Leu Lys Ser Ala Val Ser Asp Asn Gly Asn Ala 130 140

Leu Glu Glu Asp Ser Phe Arg Gln Leu Glu Lys Glu Val Lys Asp Lys 145 150 155 160

Val Lys Thr Ala Arg Met Met Ile Val Glu Ser Lys Glu Ser Tyr Asp 165 170 175

Thr Gln Leu Lys Ile Gln Lys Leu Lys Asp Thr Ile Phe Ala Val Gln 180 185

Glu Gln Leu Thr Lys Ala Lys Lys Asn Gly Ala Val Ala Ser Leu Ile 195 200 205

Ser Ala Lys Ser Val Pro Lys Ser Leu His Cys Leu Ala Met Arg Leu 210 215 220

Val Gly Glu Arg Ile Ser Asn Pro Glu Lys Tyr Lys Asp Ala Pro Pro 225 230 235 240

Asp Pro Ala Ala Glu Asp Pro Thr Leu Tyr His Tyr Ala Ile Phe Ser 245 250 255 Page 34

## 14-03 WO.sequence listing.txt

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14-03 WO.sequence listing.txt 500 505 510

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Ile Gln Ile Arg Lys Gln Ala Asp Asp His Arg Ser Leu Ala Leu Ala 85 90 95
Tyr Ala Ser Tyr Ala Arg Lys Leu Lys Leu Glu Asn Ser Lys Leu Val 100 105 110
Arg Ile Phe Ala Asp Leu Ser Arg Asn Tyr Thr Asp Leu Ile Asn Lys 115 120 125
11. 12. 12. 12. 12. 12. 12. 12. 12. 12.
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Ser Val Leu Arg Gln Phe Glu Lys Glu Val Lys Glu Arg Ile Lys Met Page 37

14-03 WO.sequence listing.txt
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14-03 WO.sequence listing.txt

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Arg Ser Gly Leu Gln Leu Trp Gln Pro Gly Ala Leu Pro Pro Thr Leu 450 460

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25 2043

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#### 14-03 WO.sequence listing.txt

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14-03 WO.sequence listing.txt

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### 14-03 WO.sequence listing.txt

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Leu Pro Phe Val Phe Ile Leu Thr Ala Val Val Thr Leu Glu Gly Val 50 60

Asn Lys Cys Ser Ser Ile Asp Cys Leu Gly Arg Arg Ile Gly Pro Arg 65 70 75

Leu Leu Gly Arg Val Asp Asp Ser Glu Arg Leu Ala Arg Asp Phe Tyr 85 90 95

Lys Ile Leu Asn Glu Val Ser Thr Gln Glu Ile Pro Asp Gly Leu Lys 100 105

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Tyr Asp Ala Lys Thr Phe Ala Leu Val Leu Arg Ala Met Met Glu Lys 130 135

Phe Glu Arg Asp Met Arg Glu Ser Lys Phe Ala Glu Leu Met Asn Lys 145 150 160

His Phe Ala Ala Ser Ser Ile Pro Lys Gly Ile His Cys Leu Ser Leu 165 170 175

Arg Leu Thr Asp Glu Tyr Ser Ser Asn Ala His Ala Arg Arg Gln Leu 180 185

Pro Ser Pro Glu Phe Leu Pro Val Leu Ser Asp Asn Ala Tyr His His 195 200 205

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Ser Ala Val Gln Ser Ser Ser Lys Pro Glu Lys Ile Val Phe His Ile 225 230 235

14-03 WO.sequence listing.txt

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Leu Arg Phe Val Phe Val Leu Ser Thr Val Asp Thr Ile Asp Gly Asp 50 60
Pro Ser Pro Cys Ser Ser Leu Ala Cys Leu Gly Lys Arg Leu Lys Pro 65 70 80
Lys Leu Leu Gly Arg Arg Val Asp Ser Gly Asn Val Pro Glu Ala Met 85 90 95
Tyr Gln Val Leu Glu Gln Pro Leu Ser Glu Gln Glu Leu Lys Gly Arg 100 105 110
Ser Asp Ile Pro Gln Thr Leu Gln Asp Phe Met Ser Glu Val Lys Arg 115 120 125
Ser Lys Ser Asp Ala Arg Glu Phe Ala Gln Lys Leu Lys Glu Met Val 130 135 140
Thr Leu Met Glu Gln Arg Thr Arg Thr Ala Lys Ile Gln Glu Tyr Leu 145 150 150
Tyr Arg His Val Ala Ser Ser Ser Ile Pro Lys Gln Leu His Cys Leu 165 170 175
Ala Leu Lys Leu Ala Asn Glu His Ser Ile Asn Ala Ala Ala Arg Leu Page 51

14-03 WO.sequence listing.txt 180 185 190

Gln Leu Pro Glu Ala Glu Leu Val Pro Met Leu Val Asp Asn Asn Tyr 195 200 205 Phe His Phe Val Leu Ala Ser Asp Asn Ile Leu Ala Ala Ser Val Val 210 220 Ala Lys Ser Leu Val Gln Asn Ala Leu Arg Pro His Lys Ile Val Leu 225 230 235 240 His Ile Ile Thr Asp Arg Lys Thr Tyr Phe Pro Met Gln Ala Trp Phe 245 250 255 Ser Leu His Pro Leu Ser Pro Ala Ile Ile Glu Val Lys Ala Leu His 260 265 270 His Phe Asp Trp Leu Ser Lys Gly Lys Val Pro Val Leu Glu Ala Met 275 280 285 Glu Lys Asp Gln Arg Val Arg Ser Gln Phe Arg Gly Gly Ser Ser Val 290 295 Ile Val Ala Asn Asn Lys Glu Asn Pro Val Val Val Ala Ala Lys Leu 305 310 315 320 Gln Ala Leu Ser Pro Lys Tyr Asn Ser Leu Met Asn His Ile Arg Ile 325 330 335 His Leu Pro Glu Leu Phe Pro Ser Leu Asn Lys Val Val Phe Leu Asp 340 345 Asp Asp Ile Val Ile Gln Thr Asp Leu Ser Pro Leu Trp Asp Ile Asp 355 360 365 Met Asn Gly Lys Val Asn Gly Ala Val Glu Thr Cys Arg Gly Glu Asp 370 380 Lys Phe Val Met Ser Lys Lys Phe Lys Ser Tyr Leu Asn Phe Ser Asn 385 390 395 400Pro Thr Ile Ala Lys Asn Phe Asn Pro Glu Glu Cys Ala Trp Ala Tyr 405 410 415 Gly Met Asn Val Phe Asp Leu Ala Ala Trp Arg Arg Thr Asn Ile Ser 420 425 430

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14-03 WO.sequence listing.txt
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His Val Gln Thr Ile Asp Pro Phe Trp His Met Leu Gly Leu Gly Tyr 465 470 480

Gln Glu Thr Thr Ser Tyr Ala Asp Ala Glu Ser Ala Ala Val His 485 490 495

Phe Asn Gly Arg Ala Lys Pro Trp Leu Asp Ile Ala Phe Pro His Leu 500 505 510

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#### 14-03 WO.sequence listing.txt

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Ser Asp Tyr Ser Ser Phe Arg Glu Ser Pro Met Phe Arg Asn Ala Glu 50 60

Gln Cys Arg Ser Ser Gly Glu Asp Ser Gly Val Cys Asn Pro Asn Leu 65 70 75

Val His Val Ala Ile Thr Leu Asp Ile Asp Tyr Leu Arg Gly Ser Ile 85 90 95

Ala Ala Val Asn Ser Ile Leu Gln His Ser Met Cys Pro Gln Ser Val 100 105 110

Phe Phe His Phe Leu Val Ser Ser Glu Ser Gln Asn Leu Glu Ser Leu 115 120 125

Ile Arg Ser Thr Phe Pro Lys Leu Thr Asn Leu Lys Ile Tyr Tyr Phe 130 140

Ala Pro Glu Thr Val Gln Ser Leu Ile Ser Ser Ser Val Arg Gln Ala 145 150 155 160

Leu Glu Gln Pro Leu Asn Tyr Ala Arg Asn Tyr Leu Ala Asp Leu Leu 165 170 175

Glu Pro Cys Val Lys Arg Val Ile Tyr Leu Asp Ser Asp Leu Val Val 180 185 190 Page 54

PCT/US2004/003545 WO 2004/072250

#### 14-03 WO.sequence listing.txt

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1038 DNA

Arabidopsis thaliana

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#### 14-03 WO.sequence listing.txt

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Ile Pro Ser Asp His Glu His Asn Pro Ser Ile Ile His Ile Ala Met 50 60

Thr Leu Asp Ala Ile Tyr Leu Arg Gly Ser Val Ala Gly Val Phe Ser 65 70 75 80

Val Leu Gln His Ala Ser Cys Pro Glu Asn Ile Val Phe His Phe Ile 85 90 95

Ala Thr His Arg Arg Ser Ala Asp Leu Arg Arg Ile Ile Ser Ser Thr 100 105 110

Phe Pro Tyr Leu Thr Tyr His Ile Tyr His Phe Asp Pro Asn Leu Val 115 120 125 Page 56

#### 14-03 WO.sequence listing.txt

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Val His Val Ala Met Thr Leu Asp Ala Ala Tyr Ile Arg Gly Ser Val 65 70 75 \_\_ Page 58

Arabidopsis thaliana <213>

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14-03 WO.sequence listing.txt 330 335 325

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900

960

1020

<210>

Met Ser Ser Arg Phe Ser Leu Thr Val Val Cys Leu Ile Ala Leu Leu 10 15 Page 60

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PRT <212> Arabidopsis thaliana

<sup>&</sup>lt;400> 38

14-03 WO.sequence listing.txt

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14-03 WO.sequence listing.txt 260 265 270

Lys Leu Glu Gln Trp Met Gln Leu Gln Lys Lys Met Arg Ile Tyr Asp 275 280 285

Leu Gly Ser Leu Pro Pro Phe Leu Leu Val Phe Ala Gly Asn Val Glu 290 295 300

Ala Ile Asp His Arg Trp Asn Gln His Gly Leu Gly Gly Asp Asn Ile 305 310 320

Arg Gly Ser Cys Arg Ser Leu His Pro Gly Pro Val Ser Leu Leu His 325 . 330

Trp Ser Gly Lys Gly Lys Pro  ${}^{\downarrow}$ Trp Val Arg Leu Asp Glu Lys Arg Pro 340 345

Cys Pro Leu Asp His Leu Trp Glu Pro Tyr Asp Leu Tyr Lys His Lys 355 360 365

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Glu Leu Thr Asp Asp Ser Ser Phe Leu 385 390

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14-03 WO.sequence listing.txt cacgctaact tcacaaagta cttcacttca ggtttctggt ccgacccggc tttacccggt
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Gly Arg Leu Thr Ala Val Ser Ala Thr Val Gly Asn Gly Phe Asp Leu 35 40 45
Gly Ser Phe Val Glu Ala Pro Glu Tyr Arg Asn Gly Lys Glu Cys Val 50 60
Ser Gln Ser Leu Asn Arg Glu Asn Phe Val Ser Ser Cys Asp Ala Ser
65 70 75
Leu Val His Val Ala Met Thr Leu Asp Ser Glu Tyr Leu Arg Gly Ser 85 90 95
Ile Ala Ala Val His Ser Met Leu Arg His Ala Ser Cys Pro Glu Asn
100 103 110
Val Phe Phe His Leu Ile Ala Ala Glu Phe Asp Pro Ala Ser Pro Arg 115 120 125
Val Leu Ser Gln Leu Val Arg Ser Thr Phe Pro Ser Leu Asn Phe Lys
130 135 140

Val Tyr Ile Phe Arg Glu Asp Thr Val Ile Asn Leu Ile Ser Ser Ser Page 63

14-03 WO.sequence listing.txt 155 160 150 145 Ile Arg Gln Ala Leu Glu Asn Pro Leu Asn Tyr Ala Arg Asn Tyr Leu 165 170 175 Gly Asp Ile Leu Asp Pro Cys Val Asp Arg Val Ile Tyr Leu Asp Ser 180 185 Asp Ile Ile Val Val Asp Asp Ile Thr Lys Leu Trp Asn Thr Ser Leu 195 200 205 Thr Gly Ser Arg Ile Ile Gly Ala Pro Glu Tyr Cys His Ala Asn Phe 210 215 Thr Lys Tyr Phe Thr Ser Gly Phe Trp Ser Asp Pro Ala Leu Pro Gly 225 230 240 Phe Phe Ser Gly Arg Lys Pro Cys Tyr Phe Asn Thr Gly Val Met Val 245 250 255 Met Asp Leu Val Arg Trp Arg Glu Gly Asn Tyr Arg Glu Lys Leu Glu 260 265 270 Thr Trp Met Gln Ile Gln Lys Lys Lys Arg Ile Tyr Asp Leu Gly Ser 275 280 285 Leu Pro Pro Phe Leu Leu Val Phe Ala Gly Asn Val Glu Ala Ile Asp 290 295 300 His Arg Trp Asn Gln His Gly Leu Gly Gly Asp Asn Val Arg Gly Ser 305 310 315 Cys Arg Ser Leu His Lys Gly Pro Val Ser Leu Leu His Trp Ser Gly 325 330 335 Lys Gly Lys Pro Trp Val Arg Leu Asp Glu Lys Arg Pro Cys Pro Leu 340 350 Asp His Leu Trp Glu Pro Tyr Asp Leu Tyr Glu His Lys Ile Glu Arg 355 360 365 Ala Lys Asp Gln Ser Leu Phe Gly Phe Ser Ser Leu Ser Glu Leu Thr 370 375 Glu Asp Ser Ser Phe Phe 385

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### 14-03 WO. sequence listing.txt

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				actttctggt			660
				aacacagggg			720
				gtagaagagt			780
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1020 1056

Phe Ile Leu Leu Val Thr Thr Thr Met Ala Val Arg Val Gly 20 25

Val Ile Leu His Lys Pro Ser Ala Pro Thr Leu Pro Val Phe Arg Glu 35 40 45

Ala Pro Ala Phe Arg Asn Gly Asp Gln Cys Gly Thr Arg Glu Ala Asp Page 65

<sup>&</sup>lt;210> 42 <211> 351 <212> PRT <213> Arabidopsis thaliana

14-03 WO.sequence listing.txt 50 55 60

Gln Ile His Ile Ala Met Thr Leu Asp Thr Asn Tyr Leu Arg Gly Thr 65 70 75 80 Met Ala Ala Val Leu Ser Leu Leu Gln His Ser Thr Cys Pro Glu Asn 85 90 95 Leu Ser Phe His Phe Leu Ser Leu Pro His Phe Glu Asn Asp Leu Phe 100 105 110 Thr Ser Ile Lys Ser Thr Phe Pro Tyr Leu Asn Phe Lys Ile Tyr Gln 115 125 Phe Asp Pro Asn Leu Val Arg Ser Lys Ile Ser Lys Ser Ile Arg Gln 130 140 Ala Leu Asp Gln Pro Leu Asn Tyr Ala Arg Ile Tyr Leu Ala Asp Ile 145 150 160 Ile Pro Ser Ser Val Asp Arg Ile Ile Tyr Leu Asp Ser Asp Leu Val 165 170 175 Val Val Asp Asp Ile Glu Lys Leu Trp His Val Glu Met Glu Gly Lys 180 185 Val Val Ala Ala Pro Glu Tyr Cys His Ala Asn Phe Thr His Tyr Phe 195 200 205 Thr Arg Thr Phe Trp Ser Asp Pro Val Leu Val Lys Val Leu Glu Gly 210 220 Lys Arg Pro Cys Tyr Phe Asn Thr Gly Val Met Val Val Asp Val Asn 225 230 235 Lys Trp Arg Lys Gly Met Tyr Thr Gln Lys Val Glu Glu Trp Met Thr 245 250 255 Ile Gln Lys Gln Lys Arg Ile Tyr His Leu Gly Ser Leu Pro Pro Phe 260 265 270 Leu Leu Ile Phe Ala Gly Asp Ile Lys Ala Val Asn His Arg Trp Asn 275 280 285 Gln His Gly Leu Gly Gly Asp Asn Phe Glu Gly Arg Cys Arg Thr Leu 290 295 300

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His Pro Gly Pro Ile Ser Leu Leu His Trp Ser Gly Lys Gly Lys Pro 305 310 315

Trp Leu Arg Leu Asp Ser Arg Lys Pro Cys Ile Val Asp His Leu Trp 325 330 335

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14-03 WO.sequence listing.txt

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Asp Ser Phe Asp Asp Ala Ser Ser Asp Leu Met Glu Ala Pro Ala Tyr 35 40 45

Gln Asn Gly Leu Asp Cys Ser Val Leu Ala Lys Asn Arg Leu Leu Leu 50 60

Ala Cys Asp Pro Ser Ala Val His Ile Ala Met Thr Leu Asp Pro Ala 65 70 75

Tyr Leu Arg Gly Thr Val Ser Ala Val His Ser Ile Leu Lys His Thr 85 90 95

Ser Cys Pro Glu Asn Ile Phe Phe His Phe Ile Ala Ser Gly Thr Ser 100 105 110

Gln Gly Ser Leu Ala Lys Thr Leu Ser Ser Val Phe Pro Ser Leu Ser 115 120 125

Phe Lys Val Tyr Thr Phe Glu Glu Thr Thr Val Lys Asn Leu Ile Ser 130 140

Ser Ser Ile Arg Gln Ala Leu Asp Ser Pro Leu Asn Tyr Ala Arg Ser 145 150 160

Tyr Leu Ser Glu Ile Leu Ser Ser Cys Val Ser Arg Val Ile Tyr Leu 165 170 175

Asp Ser Asp Val Ile Val Val Asp Asp Ile Gln Lys Leu Trp Lys Ile 180 185

Ser Leu Ser Gly Ser Arg Thr Ile Gly Ala Pro Glu Tyr Cys His Ala 195 200 205

Asn Phe Thr Lys Tyr Phe Thr Asp Ser Phe Trp Ser Asp Gln Lys Leu 210 220

Ser Ser Val Phe Asp Ser Lys Thr Pro Cys Tyr Phe Asn Thr Gly Val 225 230 240

Met Val Ile Asp Leu Glu Arg Trp Arg Glu Gly Asp Tyr Thr Arg Lys 245 255 Page 68

## 14-03 WO.sequence listing.txt

Ile Glu Asn Trp Met Lys Ile Gln Lys Glu Asp Lys Arg Ile Tyr Glu 260 265 270

Leu Gly Ser Leu Pro Pro Phe Leu Leu Val Phe Gly Gly Asp Ile Glu 275 280 285

Ala Ile Asp His Gln Trp Asn Gln His Gly Leu Gly Gly Asp Asn Ile 290 295 300

Val Ser Ser Cys Arg Ser Leu His Pro Gly Pro Val Ser Leu Ile His 305 310 315

Trp Ser Gly Lys Gly Lys Pro Trp Val Arg Leu Asp Asp Gly Lys Pro 325 335

Cys Pro Ile Asp Tyr Leu Trp Ala Pro Tyr Asp Leu His Lys Ser Gln 340 350

Arg Gln Tyr Leu Gln Tyr Asn Gln Glu Leu Glu Ile Leu 355 360 365

Arabidopsis thaliana <400> 45

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### 14-03 WO.sequence listing.txt

ggtttaggag gagataattt	tagaggactg	tgtcgagatt	tgcatccagg	tccagtgagt	900
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<213> Arabidopsis thaliana

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Asn Ser Pro Glu Cys Leu Thr Ile Glu Asn Asp Glu Asp Phe Val Cys 35 40 45

Ser Asp Lys Ala Ile His Val Ala Met Thr Leu Asp Thr Ala Tyr Leu 50 60

Arg Gly Ser Met Ala Val Ile Leu Ser Val Leu Gln His Ser Ser Cys 75 80 .

Pro Gln Asn Ile Val Phe His Phe Val Thr Ser Lys Gln Ser His Arg 85 90 95

Leu Gln Asn Tyr Val Val Ala Ser Phe Pro Tyr Leu Lys Phe Arg Ile 100 105 110

Tyr Pro Tyr Asp Val Ala Ala Ile Ser Gly Leu Ile Ser Thr Ser Ile 115 120 125

Arg Ser Ala Leu Asp Ser Pro Leu Asn Tyr Ala Arg Asn Tyr Leu Ala 130 135

Asp Ile Leu Pro Thr Cys Leu Ser Arg Val Val Tyr Leu Asp Ser Asp 145 150 160

Leu Ile Leu Val Asp Asp Ile Ser Lys Leu Phe Ser Thr His Ile Pro 165 170 175

Thr Asp Val Val Leu Ala Ala Pro Glu Tyr Cys Asn Ala Asn Phe Thr 180 185 190 Page 70

# 14-03 WO.sequence listing.txt

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Leu Ser Leu Asn Arg Arg Ala Thr Pro Cys Tyr Phe Asn Thr Gly Val 210 220

Met Val Ile Glu Leu Lys Lys Trp Arg Glu Gly Asp Tyr Thr Arg Lys 225 230 240

Ile Ile Glu Trp Met Glu Leu Gln Lys Arg Ile Arg Ile Tyr Glu Leu 245 250 255

Gly Ser Leu Pro Pro Phe Leu Leu Val Phe Ala Gly Asn Ile Ala Pro 260 265 270

Val Asp His Arg Trp Asn Gln His Gly Leu Gly Gly Asp Asn Phe Arg 275 280 285

Gly Leu Cys Arg Asp Leu His Pro Gly Pro Val Ser Leu Leu His Trp 290 295 300

Ser Gly Lys Gly Lys Pro Trp Val Arg Leu Asp Asp Gly Arg Pro Cys 305 310 315

Pro Leu Asp Ala Leu Trp Val Pro Tyr Asp Leu Leu Glu Ser Arg Phe 325 330 335

Asp Leu Ile Glu Ser 340

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### 14-03 WO.sequence listing.txt

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	tcaacacggg					780
	tgatagagaa					840
	cgccgttctt					900
						960
	atgggcttgg					1020
	gcttgcttca					
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Arabidopsis thaliana

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Ile Arg Ser Ser His Leu Asp Ala Tyr Leu Arg Phe Pro Ser Ser Asp 40 45

Pro Pro Pro His Arg Phe Ser Phe Arg Lys Ala Pro Val Phe Arg Asn 50 60

Ala Ala Asp Cys Ala Ala Ala Asp Ile Asp Ser Gly Val Cys Asn Pro 65 70 75

Ser Leu Val His Val Ala Ile Thr Leu Asp Phe Glu Tyr Leu Arg Gly 85 90 95

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Ser Val Phe Phe His Phe Leu Val Ser Glu Thr Asp Leu Glu Ser Leu 115 120 125

14-03 WO.sequence listing.txt

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130 140 Pro Glu Ile Val Arg Thr Leu Ile Ser Thr Ser Val Arg Gln Ala Leu 145 150 160 Glu Gln Pro Leu Asn Tyr Ala Arg Asn Tyr Leu Ala Asp Leu Leu Glu 165 170 175 Pro Cys Val Arg Arg Val Ile Tyr Leu Asp Ser Asp Leu Ile Val Val 180 185 190 Asp Asp Ile Ala Lys Leu Trp Met Thr Lys Leu Gly Ser Lys Thr Ile 195 200 205 Gly Ala Pro Glu Tyr Cys His Ala Asn Phe Thr Lys Tyr Phe Thr Pro 210 220 Ala Phe Trp Ser Asp Glu Arg Phe Ser Gly Ala Phe Ser Gly Arg Lys 225 230 235 Pro Cys Tyr Phe Asn Thr Gly Val Met Val Met Asp Leu Glu Arg Trp 245 250 255 Arg Arg Val Gly Tyr Thr Glu Val Ile Glu Lys Trp Met Glu Ile Gln 265 270 Lys Ser Asp Arg Ile Tyr Glu Leu Gly Ser Leu Pro Pro Phe Leu Leu 275 285 Val Phe Ala Gly Glu Val Ala Pro Ile Glu His Arg Trp Asn Gln His 290 295 300 Gly Leu Gly Gly Asp Asn Val Arg Gly Ser Cys Arg Asp Leu His Pro 305 310 315 Gly Pro Val Ser Leu Leu His Trp Ser Gly Ser Gly Lys Pro Trp Phe 325 330 Arg Leu Asp Ser Arg Arg Pro Cys Pro Leu Asp Thr Leu Trp Ala Pro 340 345 Tyr Asp Leu Tyr Gly His Tyr Ser Arg 355 360

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14-03 WO.sequence listing.txt

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			cgtggcgatc				240
			ccttcagcac				300
			aacaaacctg				360
			cgattttgcc				420
			gcagcctctg				480
			tgtcatatac				540
			tagcctaggc				600
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						agatcttaag	720
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<213> Arabidopsis thaliana

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Ile Arg Ser Ser Pro Ser Pro Ile Phe Arg Lys Ala Pro Ala Val Phe 35

Asn Asn Gly Asp Glu Cys Leu Ser Ser Gly Gly Val Cys Asn Pro Ser 50 60

14-03 WO.sequence listing.txt
Leu Val His Val Ala Ile Thr Leu Asp Val Glu Tyr Leu Arg Gly Ser
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14-03 WO.sequence listing.txt

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Thr Pro Tyr Asp Leu Tyr Arg His Ser His 340